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COARCTATION OF THE AORTA WITH UNEQUAL BLOOD PRESSURE IN THE ARMS

Case Report

RALPH G. HILLS

From The Department of Medicine, The Johns Hopkins Hospital and Medical School

Submitted for publication February 2, 1938

In his recent report on coarctation of the aorta, King (1) cited records of several cases in which the systolic blood pressure was normal in the left arm, while a marked hypertension existed in the right arm. I wish to report briefly a case of this type and to discuss a possible anatomical explanation of the phenomenon.

A. G., a white, schoolboy, 16 years of age, presented himself for physical examination before returning to school. The family history and the past history were not significant. He was a well nourished, apparently healthy boy. Examination of the head, eyes, ears, nose and throat was negative. The chest was symmetrical. There were no abnormal pulsations. The lungs were clear. Heart: the apex impulse was seen and felt in the fifth interspace 7 cm. from the mid-sternal line. The left cardiac border was 8 cm., and the right border 2 cm. from the midline in the fourth interspace. Retromanubrial dullness was 4.5 cm. in width. A faint systolic murmur heard at the apex was more intense in the pulmonic area. The second aortic sound was occasionally split. There was a systolic murmur in the aortic area which was transmitted into the vessels of the neck. The systolic murmur at the base of the heart was heard best when the patient was in the prone position. The aortic second was louder than the pulmonic second sound. In both of these areas the sounds were quite loud. The radial pulses, although synchronous, were of unequal strength, the left being smaller than the right. The blood pressure in the right arm was 180/100; in the left arm, 110/85, and in the legs, 110/90. The inequality of the blood pressure in the two arms was repeatedly verified and was noted when care had been taken to have both arms at the same level. The abdomen, genitalia and extremities showed no abnormalities.

Laboratory examinations showed that the blood counts, hemoglobin concentration and blood chemistry were normal. The blood Wassermann reaction was negative. The phenolsulphonphthalein excretion was 70% in two hours. The urinalysis showed a specific gravity of 1.018, albumin 1+, sugar 0; microscopic examination showed an occasional granular cast and white blood cell. The stool

examination was negative. The dilution-concentration test showed a specific gravity variation of 1.004 to 1.020. Examination of the nose revealed bilateral infection of the maxillary sinuses, which were later opened and drained. Following this operation the albumin disappeared from the urine. An x-ray picture of the chest showed that the arch of the aorta was increased in width, but the ascending portion of the arch could not be clearly visualized under the fluoroscope. In the x-ray pictures it was noted that there was no cervical rib to account for the inequality of the blood pressure in the two arms. The ribs showed moderate notching of the type seen in cases of coarctation of the aorta.

Diagnosis: Coarctation of the Aorta.

It was of particular interest in this case to explain the inequality of the blood pressure in the two arms. It was postulated that the anatomical defect which had resulted in a narrowing of the aorta at the foetal isthmus must extend toward the heart to include the left subclavian artery. There was, however, no precedent for this view. Unequal blood pressure in the two arms occurs normally. There is no uniformity of opinion as to how great the inequality may be. The position of the arm in relation to the heart alters the apparent blood pressure markedly. The latter may vary by as much as 40 mm. Hg. when the arm is lowered from the vertically upward to the vertically downward position (2). If the examiner unwittingly takes the pressure in the two arms in different positions, he is apt to record striking differences in pressure. Korns and Guinard (3) reported an average difference of 14.3 mm. Hg. in the two arms with a maximum of 38 mm. Hg. Some of their differences may have been due to disease. For practical purposes 38 mm. Hg. may be taken as the maximal normal variation if both antecubital fossae are at the level of the heart.

The matter was discussed with Dr. John T. King, Jr. who had found one report in the literature of involvement of the left subclavian artery in a case of coarctation of the aorta. D. E. Bedford, on hearing of Turkington's (4) case, recalled having seen a specimen in the London Heart Hospital in which the constriction had so narrowed the left subclavian artery as to admit only a large probe. Other cases of unequal blood pressure, but without autopsy reports, have been recorded by Hesdorffer (5), Blackford (6), and Denke (7). I was able to find an additional case reported by Lesselier (8) in 1882. In this case there was a narrowing of the aorta, starting at the top of the arch

and gradually increasing to a point of almost complete constriction at a level just above the insertion of the ductus arteriosus. The narrowing included the stomata of the left carotid and the left subclavian artery. Lesselier had no sphygmomanometer but he made sphygmographic tracings of the two pulses which showed the amplitude of the right radial pulse to be twice that of the left. Thus, anatomical narrowing of the mouth of the left subclavian artery does occur occasionally in coarctation of the aorta, and it seems reasonable to assume that this is the explanation of the occasional finding in such cases of normal blood pressure in the left arm associated with marked hypertension in the right arm.

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THE CEVITAMIC ACID CONTENT OF FETAL BLOOD

CONSTANTINO P. MANAHAN AND N. J. EASTMAN

From the Department of Obstetrics, Johns Hopkins University and Hospital

Submitted for publication February 26, 1938

It is generally believed that all crystalline substances of low molecular weight pass readily through the mammalian placenta and assume approximately equal concentrations in fetal and maternal bloods (1). Since cevitamic acid is a crystalline substance which resembles in its chemical configuration the pentose and hexose sugars, it would be expected to pass through the placenta, like glucose, by simple osmosis and follow this general rule. Braestrup (2), however, has recently reported that the concentration of cevitamic acid in fetal blood is several times that of the maternal blood; his observations were made on Danish women by means of the methylene blue technique of Lund and Lieck (3). With the possible exception of inorganic phosphorus, no other substance is known to exist in fetal blood in concentrations which are even twice those met with in the mother. The findings of Braestrup, accordingly, represent a singular circumstance and one that deserves further study.

The purpose of the present paper is to confirm, if possible, the observations of Braestrup over a wide range of maternal cevitamic acid levels. This has been made possible: (a) by including in the series of cases studied a number of pregnant negresses who, in Baltimore at least, often show very low cevitamic acid levels; and (2) by administering orange juice antenatally to a group of selected patients in order to raise their blood concentrations to high levels.

METHODS

Twenty-five white women and the same number of negresses, all taken at random, were subjected to venepuncture at the moment of delivery, the blood being transferred at once to bottles containing sodium oxalate and potassium cyanide, as recommended by Pijoan and Klemperer (4). Fetal blood samples, obtained by venepuncture of the umbilical vein, were treated in the same manner. Within ten minutes the two samples were analyzed for cevitamic acid by the method of Tillmans, Hirsch and Hirsch (5), as described by Eddy and Dalldorf (6).

To ten additional patients 100 cc. of orange juice were administered three times daily during the last few weeks of the prenatal period and the two bloods analyzed at delivery for cevitamic acid by the above procedure.

RESULTS

It may be seen from Fig. I that the cevitamic acid concentration of fetal blood is always several times that of the maternal blood

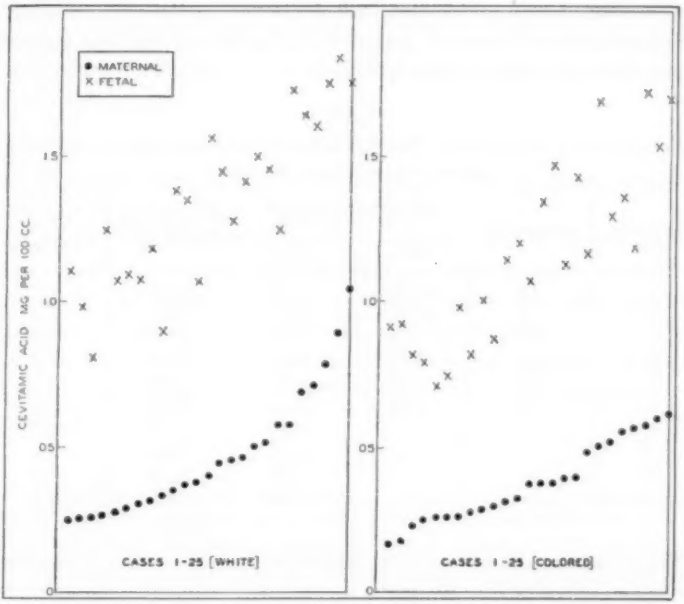

FIG. 1

whether the maternal level is high or low. In the twenty-five negresses the maternal concentration ranged from 0.17 to 0.62 mg. per 100 cc. of plasma, with a mean of 0.38 mg. ± 0.0173; the figures for the corresponding fetal plasma varied between 0.71 mg. to 1.73 mg. per 100 cc. of plasma, with a mean of 1.15 mg. ± 0.039. The concentration in the fetal plasma averaged 2.9 times that of the maternal. In the twenty-five white women the maternal concentration of cevitamic acid ranged from 0.25 mg. to 1.0 mg. per 100 cc. of plasma

with a mean of 0.47 ± 0.028; the figures for the corresponding fetal plasma varied between 0.80 and 1.84 mg. per 100 cc. of plasma with a mean of 1.34 ± 0.037. The concentration in the fetal plasma averaged 2.8 times that of the maternal.

Among the cases studied there were eight premature colored infants averaging 2022 grams in weight and three premature white infants averaging 2156 grams. In these cases the relationship between the cevitamic acid content of maternal and fetal bloods was essentially the same as that observed in mature infants; nor did the absolute figures show any appreciable difference.

TABLE 1

Showing the effect of administering 100 cc. of orange juice three times daily on cevitamic acid content of maternal and fetal bloods

CASE NUMBER	DAYS TREATED	MATERNAL CEVITAMIC ACID, MGS. PER 100 CC. PLASMA		FETAL CEVITAMIC ACID, MGS. PER 100 CC. PLASMA	RATIO FETAL / MATERNAL
		Before	After		
1	14	0.39	1.17	2.23	1.9
2	4	0.67	1.01	1.80	1.8
3	10	0.53	1.14	2.48	2.2
4	12	0.78	1.23	2.78	2.3
5	12	0.60	1.22	2.63	2.2
6	8	0.98	1.42	2.84	2.0
7			0.95	2.38	2.5
8			1.10	2.84	2.6
9			1.55	3.14	2.0
10			1.64	3.46	2.1

The results in the ten cases in which orange juice was administered are shown in Table I. As might be expected, a marked rise occurred in the cevitamic acid content of the maternal blood. Concomitantly, the concentration in the fetal blood rose to still higher levels, averaging in this group 2.1 times that of the mother.

DISCUSSION

The results of this study confirm those of Braestrup and leave little doubt that the cevitamic acid content of fetal blood is regularly two to four times that of the mother. The question arises whether the reducing substance which we analyze in fetal blood as cevitamic acid is the true vitamin or is, partly at least, some other substance

capable of reducing the phenolindophenol of the Tillmans procedure. The fact that it is possible to raise the cevitamic acid content of the fetal blood by administering orange juice to the mother speaks against such an hypothesis. Moreover, it would seem unlikely that such a substance would show, at all levels, such a constant relationship as this substance does to the cevitamic acid content of the maternal blood. This fact would likewise seem at variance with the view that the fetus synthesizes cevitamic acid. In the present state of our knowledge it seems difficult to explain this phenomenon without postulating a selective action on the part of the placenta in respect to this vitamin.

CONCLUSIONS

1. The cevitamic acid concentration in fetal blood is regularly almost three times that in the maternal blood.

2. Raising the concentration of cevitamic acid in the maternal blood by the antenatal administration of orange juice raises concomitantly the concentration in the fetal blood, the ratio between the concentrations in the two bloods remaining similar.

3. The high relative concentration of cevitamic acid in fetal blood suggests that the placenta exerts a selective action in respect to this vitamin.

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STUDIES CONCERNING HYPERTENSION IN CHILDHOOD*

I. The Development of Essential Hypertension under Observation

HELEN B. TAUSSIG, M.D. and MANES S. HECHT, M.D.

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Submitted for publication December 9, 1937

Blood pressure determinations[1] have been recorded as a routine procedure in our Cardiac Clinic since its foundation in 1928. This routine procedure has led to the recognition of a number of cases of hypertension and has afforded the unique opportunity to observe the development of essential[2] hypertension. This phenomenon has occurred in three cases of children suffering from acute rheumatic fever and chorea. The first two children have definitely developed essential hypertension and the third appears to be doing so.

The purpose of this paper is to report these cases.

Case 1. S. T. (H. L. H. No. 57314) Negro girl. Born August 28, 1919.

Family history as obtained by direct questioning was essentially negative. Both parents were living and well. There were ten siblings, three of whom had died in infancy; the remaining seven were living and well and had no complaints.

Physical examination of all the living members of the family revealed the following positive findings: The *father*, age 48, had a blood pressure of 130/85. The *mother's* blood pressure was normal, 110/80. One *brother*, age 21, had a blood pressure

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* Preliminary report read before The Society of Pediatric Research at Atlantic City, May 1936.

[1] The technique used in recording blood pressure is given in detail in the following paper.

[2] By "essential" hypertension is meant a persistent elevation of the blood pressure for which no recognized cause can be found, *i.e.*, no coarctation of the aorta, endocrine disturbance, evidence of acute or chronic nephritis, or impairment of renal function. Furthermore, in this clinic "essential" hypertension implies that there is no evidence of arteriolar disease or congenital malformation of the kidney.

between 160/90 and 150/80. A *sister*, age 19, showed slight cardiac enlargement (confirmed by X-ray). A *brother*, age 13, had rheumatic heart disease. A *sister*, age 6, had a blood pressure of 110/90.

Past history: The patient had pertussis and influenza at 4 years, chickenpox at 7 years and measles at 8 years.

She was first brought to the Harriet Lane Home in April 1928, when, at the age of 8¾ years, she had developed her first attack of chorea. At this time she was markedly underweight and gave the history of suffering from mild joint pains. This attack of chorea, which was moderately severe, lasted until August 1928. That autumn she was referred to the Cardiac Clinic.

Course under observation: In November 1928 tonsillectomy was performed. In August 1929 she had her second attack of chorea. Two months after this attack, when just 10 years of age, her blood pressure was 105/90. Thereafter it returned to normal. In the autumn of 1930 she had a third attack of chorea and in the spring of 1931 she had another recurrence. These attacks were not very severe but of such long duration that the disease appeared to be virtually chronic. The blood pressure, however, remained normal until September 1931 when it again rose—this time to 120/95. During 1931–32 she had two more prolonged attacks of chorea. At the time of puberty, in spite of these recurring attacks of chorea, the child gained weight rapidly and the level of the blood pressure fluctuated widely, at one time reaching 125/100.

In the spring of 1932 she had her sixth attack of chorea, for which she was given typhoid vaccine therapy. Although this treatment produced no immediate change in her condition, during the ensuing months she did improve and the diastolic level of the blood pressure returned to normal. In the summer of 1933 she suffered from rheumatic myocarditis, with definite signs of a mitral insufficiency, and hypertension. It was, however, not until October 1934, when she was 15 years of age, that the blood pressure reached 150/110 and it became apparent that she had developed essential hypertension. In May 1935 she had a seventh attack of chorea and the following winter she again suffered from joint pains.

In January 1936 she was admitted to the Medical Service.

Status at that time: An obese negro girl. Height 64 inches. Weight 150 pounds. The blood pressure was 140/100. The heart showed no enlargement and only a systolic murmur over the pulmonary area. The eyegrounds revealed very slight, indeed only questionable, arteriovenous compression. The urine showed a specific gravity of 1.025 but no albumin; microscopic examination of the sediment was negative. The nonprotein nitrogen of the blood was 24 mg. per cent. Phenolsulphonephthalein excretion was 65 per cent in the first hour. The urea clearance was 87 per cent of normal and 90 per cent of normal in two one-hour periods. The patient, in addition to the hypertension, however, still had a persistent low grade fever and an increased sedimentation rate. Figure 1 shows the gradual rise in the blood pressure.

Case 2. R. B. (H. L. H. No. 27561) Negro Boy. Born January 25, 1921.

Family history: Nothing is known about the father. There are two half siblings who are living and well.

The *mother's history* is of great interest. She stated that at 15 years of age she suffered from "St. Vitus' Dance." She was first seen on the Johns Hopkins Hospital Obstetrical Service in 1919 at 17 years of age when she was three months pregnant. At that time her blood pressure was 120/80. She was followed in the Obstetrical Clinic and when seven months pregnant she developed severe eclampsia and was delivered of a premature stillborn baby. In November 1920 she returned to the Obstetrical Clinic, seven months pregnant. The blood pressure and urine were both found to be normal. During the next two months she developed a nephritic toxemia without convulsions; she was delivered of a normal child (*i.e.*, R. B.). Her third pregnancy was terminated on the Obstetrical Service because of a severe toxemia. The fourth pregnancy was, according to her report, "uncomplicated" and she was delivered at home by a midwife. During the fifth pregnancy in 1928 she was again cared for on the Obstetrical Service. She again suffered from a severe toxemia of pregnancy; she was delivered by Caesarian section and sterilized. During the next nine years she was followed intermittently on the Medical Service because of hypertension. When examined in March 1937, at the age of 34 years, her heart was enlarged; eyegrounds showed early arteriolar changes; her blood pressure when first taken was 210/137 and, after resting, it fell to 144/112. In May 1937 she died of a cerebral accident.

Patient's past history: He had measles at 3 years of age. In November 1924 at 3¾ years of age he was brought to the Harriet Lane Home suffering from his first attack of chorea. The following month he was admitted to the hospital because of fever and a severe sore throat of unknown etiology.[3] In May 1924 he complained of pains in his knees.

In 1927 he had his second attack of chorea. Two years later he had a third attack of chorea and was referred to our Cardiac Clinic.

Course in Cardiac Clinic: The third attack of chorea was long and moderately severe. Bed rest was urged but never achieved. At this time his blood pressure was 110/70 and an electrocardiogram showed a P-R interval which varied between 0.22 and 0.30 of a second, and a left axis deviation. In September 1931 he suffered from a fourth attack of chorea. Two months later the chorea had subsided, but his heart showed a definite systolic murmur which was transmitted to the axilla; the conduction time was still 0.30 of a second. He was only 10 years of age. The blood pressure was 120/100. After this initial rise in blood pressure there was a period during which it showed wide fluctuations.

In February 1932 he had his fifth attack of chorea. The following year, in March 1933, when just 12 years of age, he had his sixth attack. During this attack his blood pressure reached 130/98; since then his diastolic pressure has

[3] Throat cultures at that time were negative for diphtheria and hemolytic streptococci and no Vincent's organisms could be demonstrated.

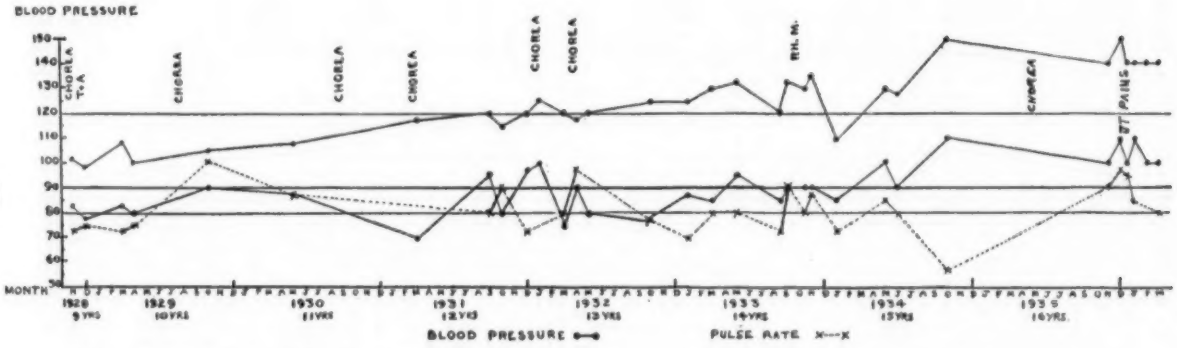

FIG. 1. BLOOD PRESSURE CHART OF S. T. (CASE 1) SHOWING GRADUAL DEVELOPMENT OF ESSENTIAL HYPERTENSION

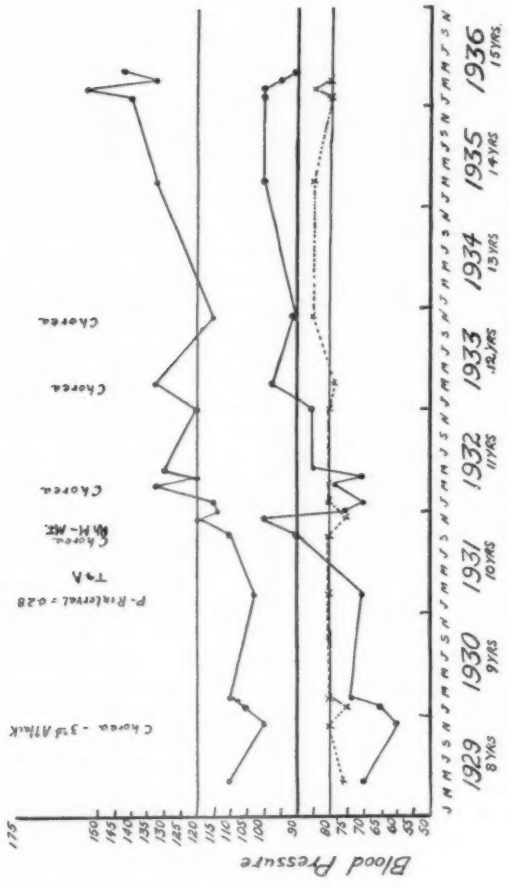

FIG. 2. BLOOD PRESSURE CHART OF R. B. (CASE 2) SHOWING GRADUAL DEVELOPMENT OF ESSENTIAL HYPERTENSION

been constantly at 90 or above. In the fall of 1933 he had his seventh attack of chorea. The course of the blood pressure is shown in Figure 2.

Status in April 1936: Well developed boy. Height 63 inches. Weight 120 pounds. Blood pressure 140/100. Examination of the heart revealed a soft systolic murmur at the apex. The teleoroentgenogram showed that the heart was normal in size. There were no arteriolar changes visible in the eyegrounds. The cold test of Hines and Brown was negative. The urine showed a concentration of 1.025. No albumin; microscopic examination of the sediment negative. The nonprotein nitrogen of the blood was 32 mg. per cent. The phenolsulphone phthalein excretion was 25 per cent in 15 minutes, 55 per cent in 1 hour. Intravenous pyelogram normal. The only positive findings were an acceleration of the sedimentation rate and a P-R interval of 0.24 to 0.28 of a second in the electrocardiogram.

Case 3. J. G. (H. L. H. No. 78041) White boy. Born September 4, 1923.

Family history, as obtained, was non-contributory. The father refused to have the family examined and only permitted us to take his own blood pressure and that of his wife. Both were normal. The mother did, however, give a history of high blood pressure during her last pregnancy. There were five siblings, two of whom had died in infancy. The others were said to be well.

Past history: The patient had whooping cough at 1½ years and scarlet fever at 6 years.

He was first seen in the Harriet Lane Home in June 1932 at the age of 8 years, at which time he was suffering from acute rheumatic fever; he was immediately referred to the Cardiac Clinic.

Course under observation: From June through August 1932 he was in bed because of acute rheumatic fever and rheumatic myocarditis. In September it proved impossible to keep him in bed and, therefore, in the belief that he would be quieter in school, he was permitted to attend.

In November 1932 he developed chorea and was put to bed. Following this attack, in January 1933 his blood pressure was 120/90. The blood pressure subsequently returned to normal and the boy remained well for a year. In January 1934 he developed a cough and a cold and the murmur in his heart increased in intensity. He was put to bed for two months. In March 1934, when beginning to get up, his blood pressure reached 130/110. He was then 10½ years of age. Between January 1934 and January 1935 he gained ten pounds and in the following two months he gained another ten pounds in weight. During this period his blood pressure fluctuated widely. In December 1935 it again reached 135/110. Since then it has continued to fluctuate but has remained definitely above normal (Fig. 3). From January 1934 to April 1936 the boy was asymptomatic.

Status in April 1936: Obese boy of 13 years. Height 57¼ inches. Weight 107 pounds. The teleoroentgenogram showed that the heart was of normal size but there was a systolic murmur at the apex. Basal metabolic rate was 0%.

Electrocardiogram normal. The erythrocyte sedimentation rate was 30 mm. in one hour. Urine examination essentially negative. S. G. 1.018; no albumin; microscopic examination of the sediment, negative. The nonprotein nitrogen of the blood done on a routine specimen was 40 mg. per cent. The phenolsulphonephthalein excretion was 35 per cent in 15 minutes, 60 per cent in one hour. The intravenous pyelogram was normal.

DISCUSSION

The family studies, in the two cases in which this was possible, revealed the existence of both hypertension and rheumatic fever. In the first case, although the parents are well, there is evidence of rheumatic fever and hypertension among the siblings. In the second case the mother is said to have suffered from St. Vitus' Dance, is known to have suffered from repeated toxemia of pregnancy and to have died at the age of 34 from hypertension and a cerebral accident. In the third case the family refused examination. Therefore, although no history of rheumatic fever or chorea was obtained, nothing definitive is known.

The rheumatic infections in these cases are most striking. All three children have suffered from chorea and acute rheumatic fever and rheumatic myocarditis and have been kept under observation because of the rheumatic illness. In the first case, the patient suffered from chorea almost constantly for three years and thereafter from rheumatic myocarditis. The second child suffered from repeated attacks of chorea and has had persistent signs of rheumatic myocarditis. The third boy suffered from both acute rheumatic fever and chorea. The first two patients unquestionably have developed essential hypertension. In the third case the hypertension is not as pronounced or as persistent as in the first two cases. Nevertheless, it became manifest at an earlier age and in relation to age it has always been more pronounced than in either of the first two cases. Therefore, it appears probable that the child is developing essential hypertension.

In each instance none of the usual causes for the hypertension could be found. The eyegrounds were normal and extensive renal investigations revealed no abnormality.

It is of interest, and probably of significance, that all three children, although clinically well, have repeatedly shown slight evidence of a

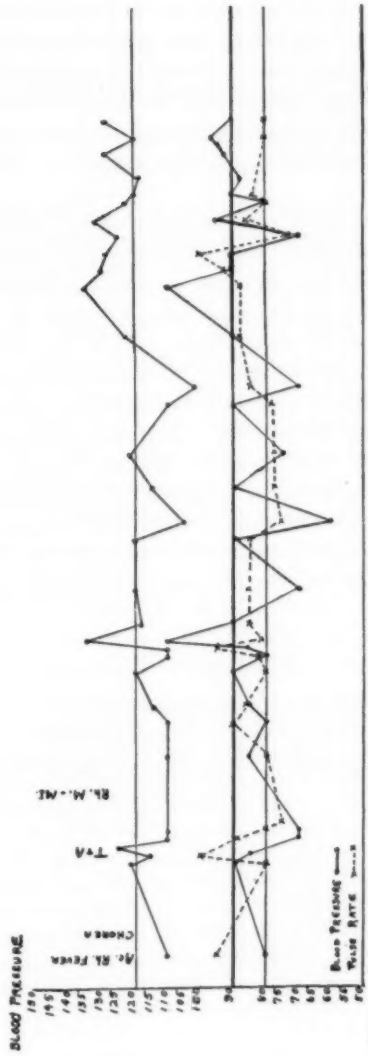

FIG. 3. CHART OF J. G. (CASE 3) SHOWING A GRADUAL RISE IN THE BLOOD PRESSURE WHICH NOW REMAINS CONSTANTLY ELEVATED

persistent infection as indicated by accelerated sedimentation rates. In addition, the first shows a persistent low grade fever and the second a persistent first degree heart block. These findings, together with the histories and the gradual development of the hypertension during the period of repeated rheumatic illnesses, strongly suggest that the hypertension is in some way related to, or exaggerated by, the rheumatic infection.

Whatever may be the etiology of the hypertension, inasmuch as the first two children had normal blood pressures when referred to our clinic, their records illustrate the way in which hypertension may develop. In both instances the first clear evidence of high blood pressure was observed at 10 years of age. After the initial peak in the blood pressure, there was an interval of several years during which there were wide fluctuations in the level of the blood pressure before the hypertension became permanent. This phenomenon illustrates that the finding of high blood pressure readings cannot be ignored because of the subsequent finding of a normal blood pressure. Indeed, a single diastolic reading of 90 mm. in a child of 10 years obtained under satisfactory conditions indicates that the child must be followed for a period of years before the diagnosis of hypertension can be excluded.

SUMMARY

Three cases are reported in which during the course of a rheumatic infection hypertension has developed under observation.

The importance of abnormally high blood pressures in young children is emphasized.

STUDIES CONCERNING HYPERTENSION IN CHILDHOOD[1]

II. The Occurrence of Hypertension in Acute Rheumatic Fever in Childhood

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The development of essential hypertension in three children while under observation because of rheumatic infections (34) led us to investigate our other cases of rheumatic fever and chorea in which high diastolic readings had been noted. Prior to this, although we had appreciated that many children with rheumatic fever and chorea showed wide fluctuation in the level of their blood pressure and diastolic pressures of 90 mm. of mercury were not infrequently recorded, no special attention had been directed toward this observation. The realization that in some cases high blood pressures were of the utmost significance led us to investigate this finding in our other patients suffering from rheumatic fever and chorea.

CRITERIA AND SELECTION OF CASES

In this study, regardless of the age of the child, a diastolic level of 90 mm. of mercury or above has been considered as indicative of hypertension. We believe the normal diastolic level for a child of 5 years is considerably lower than this. Nevertheless, since there is no certain knowledge concerning the hypertensive levels in childhood it is better to err on the side of selecting a hypertensive level that is too high rather than too low. In the majority of these cases a systolic level of 130 mm. of mercury or above has been considered abnormal, but owing to the greater significance of the diastolic level, children showing a blood pressure of 120/90 have been included in this investigation.

In accord with the best practice in recording blood pressures, the systolic level has been taken not at the very first sound, but when the sounds are heard clearly

[1] Preliminary report read before The Society of Pediatric Research at Atlantic City, May 1936.

and regularly, and the diastolic pressure is taken not at the very last sound but at the "fourth phase" or the point at which there is a definite change in the intensity of the sound (23). When trying to obtain the lowest possible diastolic reading, care must be taken not to exert undue pressure on the artery with the stethoscope because such pressure in itself constricts the artery thereby giving an artificially low reading on the mercury manometer.

In every discussion of the significance of blood pressure, the question is always raised concerning the veracity of the readings. The emotional response of the individual is always the first factor to be questioned. Therefore, owing to the excitement which is so frequently aroused by the first attempt to take the blood pressure, the readings obtained at the time of the first examination have been disregarded. The pulse rate has been used as an index of excitement; no case with a tachycardia (over 100 per minute) has been included unless similar high readings had been obtained when the pulse rate was normal (80 per minute). As a further precaution, high blood pressure readings were not considered significant of hypertension unless a note had been made at the time of examination stating that the child was quiet.

The size of the cuff also deserves special consideration. A narrow (5 cm.) cuff is generally recommended for obtaining the blood pressure in the case of children 5 to 10 years of age. It is our opinion that the width of the cuff should not be determined by the age of the child but should vary directly in proportion to the size of the arm. The blood pressure cuff should cover the middle two-thirds of the upper arm and the rubber armlet should completely encircle it. If the cuff is too narrow or the rubber too short, an artificially high reading is obtained. It is our routine practice to use three different widths of cuff varying with the size of the child. The width of the cuff is of such importance that all cases in which its size was not definitely known have been excluded from this study.

Naturally all cases were eliminated which showed any of the factors which are known to alter the level of the blood pressure, such as, acute and chronic nephritis, urinary tract infection, and impairment of renal function. Similarly all cases of aortic insufficiency were excluded and blood pressure determinations on the lower extremities were made in order to rule out coarctation of the aorta. Basal metabolic tests were performed on all cases showing persistent tachycardia or other signs suggesting hyperthyroidism. No cases with increased basal metabolic rates were included. All cases of decompensation, and even those with severely damaged hearts in which decompensation seemed imminent, were discarded.

There were 560 children with rheumatic fever or chorea registered in the Cardiac Clinic on whom four or more blood pressure readings had been recorded. Of the 560, 60 had complicating factors, such as, aortic insufficiency (37); decompensation, as evidenced by engorgement of the liver, râles at the lung bases, or edema of the extremities, or severe heart disease (17); acute nephritis (4); and

increased basal metabolic rate (2). The elimination of these cases left 500 cases to be analyzed. Of these 500, 278 of the children had normal blood pressures; 49 showed definite hypertension. The remaining 174 represent children in whom elevated blood pressure readings were recorded, but on whom there were either an insufficient number of blood pressure readings or insufficient data to exclude possible complicating factors. Therefore, in spite of its size, no great significance is to be attached to this group.

The 49 children who did show hypertension may be divided into two groups (I) those who have shown hypertension in relation to a rheumatic episode, either in association with (a) chorea alone, (b) chorea and rheumatic fever, (c) rheumatic fever alone; and (II) those who have shown repeated high blood pressure determinations but in whom the significance of the hypertension is more difficult to evaluate.

In order to exclude the possibility of renal involvement, all children who have shown hypertension and in whom the blood pressure has not returned to normal have had careful renal investigations.

The technique used in the renal examinations was as follows: A specimen of urine was examined in the routine manner in the dispensary. In all cases in which the urine showed a low specific gravity, the power of the kidney to concentrate was estimated by the specific gravity of the first specimen of urine passed in the morning immediately after the patient arose. The nonprotein nitrogen determinations were made on samples of blood taken in the afternoon. A fasting nonprotein nitrogen was determined only on those cases in which the routine determination was over 36 mg. per cent. The phenolsulphonephthalein excretion was determined by the fractional method (38) after the intravenous injection of 1 c.c. of dye.[2] Finally, roentgenograms of the kidneys were taken after the intravenous injection of neo-iopax to exclude the possibility of congenital malformations or other gross anatomical alterations in the urinary tract.

GROUP I. CASES SHOWING HYPERTENSION IN CLOSE ASSOCIATION WITH A RHEUMATIC EPISODE

This group includes those children in whom hypertension has been observed in connection with a rheumatic episode, *either* (*A*) *chorea*, (*B*) *chorea and acute rheumatic fever*, *or* (*C*) *rheumatic fever alone*. For the sake of clarity these cases have been separated in the tables (I to IV) according to the type of rheumatic episode. In these cases

[2] A Dunning colorimeter was used in these tests.

the hypertension is not considered as permanent, because in all instances either the blood pressure has returned to normal after the rheumatic infection has subsided or the rheumatic illness is still of recent date. In all of the cases in which the blood pressure has returned to normal, the hypertension was clearly a transitory phenomenon. In some cases the period of hypertension coincided with the acute stage of the illness; in others the hypertension appeared to follow in the wake of the illness.[3] Indeed, a number of cases have been observed in which several months were required after the subsidence of the acute illness before the blood pressure returned to normal. For this reason we do not believe any conclusion can be drawn concerning the recent cases in which the blood pressure is still elevated. In all of these cases the renal function was carefully investigated.

A. Occurrence in children suffering from chorea

There were five cases of hypertension observed in children suffering from chorea. The salient features of these five cases (Nos. 1 to 5) are summarized in Table I. In each instance the hypertension occurred while the child was up. In two cases (Nos. 4 and 5) the children were up against advice during the acute stage of the illness, whereas, in the other three, the period of hypertension occurred during convalescence. Case 2 is of interest because the patient showed a slight elevation of blood pressure with a diastolic level of 88 mm. after the fourth attack of chorea and a more pronounced and more prolonged elevation of blood pressure following the fifth attack. A

[3] It may be inaccurate to attempt to distinguish between "during" and "immediately following" an acute rheumatic episode, because it is notoriously difficult to know when a rheumatic infection has entirely subsided. In this study the term "immediately following" or "after" a rheumatic infection is used to cover the period of convalescence when the usual clinical and laboratory evidences of an active infection have subsided and the doctor, believing the process is inactive, has recommended that the child either increase his activity or resume a normal life. It is entirely possible, especially in the earlier years of the clinic when the test of the erythrocytic sedimentation rate was not used as an index of activity, that in some of the cases believed to be inactive in reality there still remained a mild infection. It is, indeed, conceivable that in the cases showing hypertension the blood pressure may, in some instances, be the last factor to return to normal after a rheumatic illness.

TABLE I

Hypertension in association with chorea

Att.	Attack	Ht.	Heart	Rh.F.	Rheumatic Fever
B.M.R.	Basal Metabolic Rate	Hyper.	Hypertension	Rh.M.	Rheumatic Myocarditis
B.P.	Blood Pressure	Jt.P.	Joint Pains	Rh.Poly.	Rheumatic Polyarthritis
Ch.Rh.H.D.	Chronic Rheumatic Heart Disease	M.I.	Mitral Insufficiency	S.R.	Sedimentation Rate
		Micro.	Microscopic examination	Tachy.	Tachycardia
Conval.	Convalescing	M.S.	Mitral Stenosis	W.B.	Whole Blood
Ep.C.	Epithelial Cells	Norm.	Normal	W.b.c.	White blood cells
Ery. marg.	Erythema marginatum	P.	Pulse		

CASE NO.	NAME	AGE	SEX	COLOR	RHEUMATIC HISTORY	HYPERTENSION 1ST OBSERVED		STATUS IN 1936				
						Age	B.P.*	B.P. & Ht.	Urine	N.P.N.	P.S.P.	Pyelogram
1	I. M. 71380	18	F	W	Chorea 1st att. 11 yrs. 2nd att. 13 yrs. 3rd att. 14 yrs. Hyper. after 3rd att.	14	120/90	130/80 Ht. norm. (Sept.'34)	Moved to Philadelphia			
2	J. D. 26786	15½	M	W	Chorea, 5 att. 1st att. 8 yrs. 5th att. 13½ yrs. Hyper. after 5th att.	13½	120/90	115/70 Ht. norm.	1.010 Alb. 0 Occ. casts (voided)	31	35% ½ hr. 64% 1 hr.	
3	H. S. 52958	15½	M	W	Chorea, 11 att. 1st att. 3 yrs. 11th att. 15 yrs. Hyper. after 11th att.	15½	130/90	105/70 Ht. norm.	1.027 Alb. 0 Micro.: neg.	36	38% ½ hr. 68% 1 hr.	Normal
4	L. H. 78351	13	F	W	Chorea 1st att. 8 yrs. 2nd att. 9 yrs. 3rd att. 13 yrs. Hyper. after 2nd and 3rd att.	9	110/90	120/90 Ht. norm.	1.030 Alb. 0 Micro.: neg.	35 (W.B.)	25% ½ hr. 65% 1 hr.	Normal
5	L. M. 77896	8	M	W	Chorea 1st att. 8 yrs. T. and A. 8 yrs. Hyper. 8½ yrs.	8½	135/90	110/70 Ht. norm.	1.030 Alb. 0 Micro.: neg.	At convalescent home		

* Highest reading at time hypertension was first observed.

similar phenomenon was observed in Case 4. This patient had her second attack of chorea at the age of 9 years; following this attack the blood pressure was 110/90. During her third attack of chorea in September 1935, the blood pressure rose to 150/100. Although she is now clinically well, the blood pressure still remains 120/90.

B. Occurrence in connection with chorea and rheumatic fever

There are eight children in whom hypertension occurred associated with chorea and rheumatic fever. These cases (Nos. 6 to 13) are summarized in Table II. In this group there are only two children (Cases 7 and 12) in whom the blood pressure has not returned to a definitely lower level. Case 7 is that of a child who first showed a hypertension after her second attack of chorea and still shows an elevated blood pressure. During the past winter, although clinically well, she has developed a murmur in her heart, which is presumptive evidence of recent rheumatic activity. In Case 12 the infection is still active, as evidenced by the acceleration of the sedimentation rate. In this case hypertension occurrred on the two occasions when, contrary to our advice, the boy attempted to get up.

It should be noted that in this group in only one instance (Case 10) was the patient receiving complete bed rest at the time the hypertension was observed. Case 13, however, illustrates that the hypertension does not necessarily disappear as soon as the child is put to bed. In this instance the boy had been allowed up after his second attack of chorea before he was brought to the Harriet Lane Home in September 1935. On examination the blood pressure was found to be 120/90. Therefore, even though the boy had no complaints and the sedimentation rate was normal, he was put to bed, and was not allowed up again until the spring of 1936 when the diastolic pressure had returned to 80 mm.

C. Occurrence in connection with rheumatic fever alone

For the sake of clarity we have separated these cases into two tables according to whether they were seen prior to or during the year 1935–36.

In Table III we have summarized the twelve cases (Cases 14 to 25) which were observed prior to 1935–36. All of these children now have normal blood pressures; hence in these cases the hypertension was

TABLE II

TABLE II

Hypertension in association with chorea and acute rheumatic fever

CASE NO.	NAME	AGE	SEX	COLOR	RHEUMTAIC HISTORY	HYPERTENSION 1ST OBSERVED		STATUS IN 1936				
						Age	B.P.*	B.P. & Ht.	Urine	N.P.N.	P.S.P.	Pyelogram
6	A. K. 65715	15½	F	W	Chorea 1st att. 8 yrs. 2nd att. 9 yrs. 3rd att. 11 yrs. Sl. card. enlargement 12 yrs.	12½	144/90	120/80 Ht. norm.	1.018 Alb. 0 Micro.: neg.	37 (W.B.)	30% ½ hr. 55% 1 hr.	
7	E. K. 85520	9	F	C	Chorea 1st att. 5 yrs. 2nd att. 6 yrs. Rh.M. 6½ yrs.	7	120/90	120/89 M.I.		38	37% ½ hr. 62% 1 hr.	
8	E. P. 86708	8	F	W	Chorea 1st att. 7 yrs. 2nd att. 8 yrs. Ery. marg. 8 yrs.	7	130/94	90/55 Ht. norm.				
9	E. P. 79662	11½	M	W	Chorea, 5 att. 1st att. 7 yrs. 5th att. 11 yrs. Jt.P. 1st att. 8 yrs. 2nd att. 11 yrs.	9½	135/90	110/70 Ht. norm.				
10	C. B. 63614	16	M	C	Chorea 1st att. 11 yrs. Rh.F. 1st att. 11½ yrs. Chorea 2nd att. 14 yrs.	15	135/90†	120/80 Sys. mur. at apex after exercise	1.026 Alb. 0 Micro.: neg.			
11	F. S. 65348	7	M	W	Scarlet fever 2 yrs. Rh.F. 5½ yrs. Chorea ? 6½ yrs. Hyper. 6½ yrs.	6½	110/90	100/60 Ht. norm.	1.030 Alb. 0 Micro.: neg.			
12	W. E. 40736	11	M	C	Chorea & Rh.M. 1st att. 11 yrs.	11	118/92	105/85 M.I. S.R. accel.	1.021 Alb. 0 Micro.: neg.	33	40% ½ hr. 75% 1 hr.	Normal
13	T. S. 94213	11	M	W	Rh.F. 1st att. 8 yrs. Chorea 1st att. 8½ yrs. T. and A. 8½ yrs. Chorea 2nd att. 11 yrs. Hyper. after 2nd att.	11	120/90	120/80 M.I.	1.018 Alb. +	34	37% ½ hr. 70% 1 hr.	Normal

* Highest reading at time hypertension was first observed. † Patient hospitalized because of chorea.

TABLE III

Hypertension with acute rheumatic fever, prior to 1935–36

CASE NO.	NAME	AGE	SEX	COLOR	RHEUMATIC HISTORY	HYPERTENSION 1ST OBSERVED		STATUS IN 1936				
						Age	B.P.*	B.P. & Ht.	Urine	N.P.N.	P.S.P.	Pyelogram
14	G. F. 41076	14	F	W	Jt.P. 11–12 yrs. 1st seen 12 yrs. Rh.M. P-R 0.20 B.M.R. + 1	12	140/90	125/70 Ht. norm.	1.020 Alb. 0 Micro.: neg.	33.3	35% ½ hr. 75% 1 hr.	Normal
15	J. R. 87497	12	M	W	Rh.F. 1st att. 7 yrs. 2nd att. 10 yrs. Rh.M., M.I. 10 yrs. (Never stayed in bed)	10	130/90	115/70 Ht. norm.		37	30% ½ hr. 70% 1 hr.	
16	L. E. 43137	14	M	W	Rh.F. 1st att. 8 yrs. Rh.M. & M.I. 2nd att. 13 yrs. Hyper. 13 yrs. (in bed)	13	112/92	112/68 Ht.: sl. enlarged. M.I. & M.S.?	1.018 Alb. 0 Micro.: neg.	35	20% ½ hr. 70% 1 hr.	
17	R. R. 81247	15	F	W	Rh.F., Rh.Poly. 1st att. 12 yrs. Ekg.: T diphasic in L_2 Hyper. following Rh.F.	12	130/90	120/80 Ht. norm.	1.020 Alb. 0	38	50% ½ hr. 65% 1 hr.	
18	D. S. 80012	11	F	W	Rh.F., Pancarditis 1st att. 7–8 yrs. Hyper. 8½ yrs. Rh.M. 9 yrs. Hyper. 9 & 10 yrs.	8½	120/90	120/70 Ht. norm. in size. M.I M.S.?	1.025 Alb. 0			
19	W. S. 24785	17	M	W	Rh.F. 1st att. 11 yrs. 2nd att. 13 yrs. Hyper. while conval. from 2nd att.	13½	140/100	110/80 Ht. norm.	Last seen in 1932			

20	S. R. 32847	16	M	W	Rh.F., Rh.M., M.I. 1st att. 9 yrs. 2nd att. 10½ yrs. 3rd att. 12¼ yrs. Hyper. 13 yrs. (following 3rd att.) 4th att. 16 yrs.	13	135/90	115/80 Rh.F., Rh.M. 4th att.	1.028 Alb. 0 Micro.: neg.			
21	N. R. 30653	17	M	W	Rh.F., M.I. 1st att. 9½ yrs. 2nd att. 10 yrs. 3rd att. 14 yrs. M.I., iritis, & hyper. (Pt. up)	14	130/90	110/70 Ht. norm.				
22	G. R. 89746	9	F	W	Rh.F., M.I. 1st att. 7½ yrs. Ery. marg. 7½ yrs. Hyper. c̄ 1st att. (Pt. in bed)	7¾	120/90	110/70 R.H.D. c̄ M.I.				
23	B. S. 90724	6	F	W	Rh.F., M.I. 1st att. 4¼ yrs. Hyper. c̄ 1st att. (Pt. up)	4¼	120/90	98/70 M.I.	Moved—Unable to locate			
24	P. M. 79761	13	F	W	Jt.P., M.I. ?, 11 yrs. B.M.R. +4	11	130/90	112/75† Ht. norm.	1.010 (voided) Alb. 0	Moved to Florida		
25	C.M. 65173	19	F	W	Jt.P., P. 120 B.M.R. + 11 (at 16 yrs.)	16	142/90	126/83† Ht. norm.	1.005 (voided) Alb. +	Moved to Florida		

* Highest reading at time hypertension was first observed.
† I am indebted to Dr. Harriss for these reports.

clearly a transitory phenomenon and no renal investigations were made. In only three instances (Cases 16, 18 and 22) were the patients at complete bed rest at the time the hypertension was observed. At that time, however, the finding of hypertension was not considered significant and, therefore, no effort was made to treat these children with rest in bed. The fact that these pressures have returned to normal without treatment may at first glance suggest that the phenomenon of hypertension is of no clinical importance. It must, however, be remembered that three children have developed essential

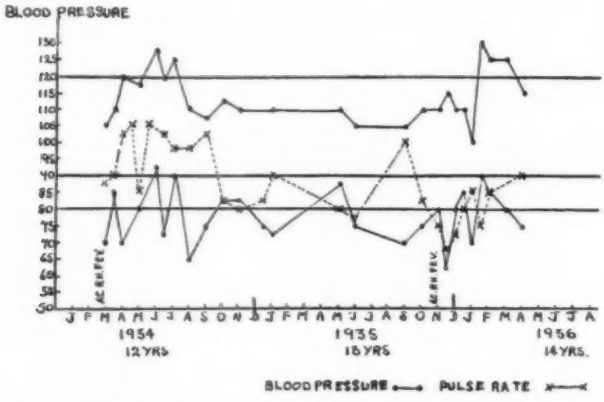

FIG. 1. CHART OF E. C. (CASE 26) SHOWING RISE OF BLOOD PRESSURE FOLLOWING EACH OF TWO ATTACKS OF ACUTE RHEUMATIC FEVER

hypertension during the course of recurrent attacks of rheumatic fever and chorea (34). Furthermore, there is the additional group of children[4] in whom repeated high blood pressures have been recorded but in whom the blood pressure has not yet stabilized.

Table IV summarizes the twelve cases which were observed during 1935–36. The large number of cases recorded during this year was undoubtedly due to the fact that we were aware of the phenomenon and consequently blood pressures were taken with greater frequency and greater care. Two cases (Nos. 26 and 34) warrant special note,—the first because the patient showed hypertension on two occasions; the second because of the severity of the hypertension.

[4] To be discussed presently.

Case 26. A negro girl of 14 years who has had two attacks of acute rheumatic fever. After each illness, upon the resumption of activity, she showed an increased blood pressure for a period of two to three months (Fig. 1). The first period of hypertension occurred when she had apparently recovered from her first attack of rheumatic fever and had been allowed to return to school. The second period of hypertension occurred following the second attack after all other signs of an active infection had subsided and she had been allowed to be up two hours daily. Upon developing hypertension, even though she showed no other indication of an active rheumatic infection, no further increase in her activity was recommended until the blood pressure had returned to normal.

Case 34. A white boy of 10 years who has a very severe and persistent hypertension. He has had two definite attacks of acute rheumatic polyarthritis. He was brought to the hospital in February 1936, following his second attack of polyarthritis, and in the routine physical examination the blood pressure was found to be 160/100. He was cared for at home for one month and then admitted to the hospital for further investigation of the hypertension. During his stay in the hospital the average blood pressure was 135/100 when awake and the lowest reading obtained while asleep was 110/85. Throughout the spring of 1936 the blood pressure varied between 130/95 and 160/110. This child was not seen prior to the onset of the present illness and has shown a persistent marked hypertension. He has not been followed for a sufficient length of time to ascertain whether there is any relation between the rheumatic infection and the hypertension.

In the remaining ten cases the hypertension occurred during an acute rheumatic episode. Three of these children (Nos. 33, 35 and 37), contrary to advice, were up. The remaining seven were in bed and remained in bed throughout their illness. None had severe cardiac involvement and in none were there any complaints referable to the hypertension.

To sum up: We have observed hypertension in close association with a rheumatic episode in 37 cases. In the majority of cases the blood pressure has returned to normal. Inasmuch as in some cases three to four months were required for the blood pressure to return to normal, the other cases are too recent to permit conclusions concerning the character of the hypertension. All of the patients in whom the blood pressure remained elevated were carefully studied.

The usual causes[5] of hypertension were readily excluded; none of these cases showed any evidence of arteriosclerosis. Furthermore,

[5] See *Criteria and Selection of Cases.*

TABLE IV

Hypertension with acute rheumatic fever, 1935–36

CASE NO.	NAME	AGE	SEX	COLOR	RHEUMATIC HISTORY	HYPERTENSION 1ST OBSERVED		STATUS IN 1936				
						Age	B.P.*	B.P. & Ht.	Urine	N.P.N.	P.S.P.	Pyelogram
26	E. C. 87247	14	F	C	Rh.F. 1st att. 12 yrs. 2nd att. 13 yrs. Hyper. after 1st and 2nd att.	12	128/92	115/75 Ht.: sys. at apex	1.014 Alb. 0	31	38% ½ hr. 58% 1 hr.	Normal
27	W. W. 72988	5	M	C	Rh.M. 4 yrs. Hyper. 4½ yrs. (during conval.)	4½	120/90	110/70 M.I.	1.018 Alb. 0	34	25% ½ hr. 55% 1 hr.	Normal
28	J. N. 52055	10	F	W	Rh.F. 1st att. 10 yrs. Hyper. c̄ 1st att. (in bed)	10	130/90	126/74 Ht. norm.		35	35% ½ hr. 80% 1 hr.	Normal
29	W. F. 94188	12¾	M	W	Rh.F. 1st att. 12 yrs. Hyper. c̄ Rh.F. (in bed)	12	138/95	138/80 M.I.	1.020 Alb. 0	32	35% ½ hr. 70% 1 hr.	Normal
30	M. B. 52488	9	F	C	Rh.F. 1st att. 9 yrs. Hyper. during 1st att. (in bed)	9	130/90	120/70 Ht.: sys. at apex	1.020 Alb. 0 Micro.: Occ. W.b.c.	28	45% ½ hr. 60% 1 hr.	Horse-shoe kidney
31	G. B. 90895	13	M	C	Rh.F. 1st att. 13 yrs. Hyper. c̄ Rh.F. (in bed)	13	135/95	105/60 M.I. ?	1.022 Alb. 0	36	30% ½ hr. 60% 1 hr.	Normal
32	R. W. 94233	11	M	W	Rh.F. 1st att. 11 yrs. Hyper. c̄ Rh.F. (in bed)	11	136/88	130/75 M.I.	1.022 Alb. 0 Micro.: neg.	34	48% ½ hr. 75% 1 hr.	Normal

33	T. S. 89999	11½	M	C	Rh.F. 1st att. 10 yrs. Rh.M. 11 yrs. Ac. tonsil. 11½ yrs. Hyper. after tonsil. (up)	11½	150/90	120/75 M.I. M.S. ?	1.022 Alb. 0 Micro.: neg.	33	45% ½ hr. 85% 1 hr.	Normal
34	J. P. 72719	10	M	W	Rh.F. 1st att. 9½ yrs. 2nd att. 10 yrs. Hyper. 10 yrs. (in bed)	10	150/100	140/100 Ht. norm.	1.022 Alb. 0	32	25% ½ hr. 60% 1 hr.	Normal
35	H. B. 86498	13	F	M†	Rh.F. 1st att. 11 yrs. 2nd att. 12 yrs. Hyper. after 2nd att. (up)	12½	120/90	130/70 M.I.	1.028 Alb. 0 Sug. 0 Micro.: neg.	32	40% ½ hr. 74% 1 hr.	
36	W. B. 94890	14	M	W	Rh.F. 1st att. 13½ yrs. Hyper. 14 yrs. (up but quiet)	14	155/90	120/70 Ht. norm.	1.026 Alb. 0 Micro.: neg.	31	30% ½ hr. 65% 1 hr.	
37	C. R. 76870	11	M	W	Rh.F. 1st att. 8 yrs. 2nd att. 10¾ yrs. Hyper. c 2nd att. (up)	10¾	120/95	120/100–110/80 Rh.F., active	1.030 Alb. tr. Micro.: neg.	35	35% ½ hr. 65% 1 hr.	

* Highest reading at time hypertension was first observed.
† Mulatto.

the renal investigations in all of these cases, as shown in Tables I to IV, were essentially negative. Addis counts were not done, but the non-protein nitrogen of the blood was not elevated and phenol-sulphonephthalein excretion was normal. Only one case showed a congenital malformation of the kidney. This child had suffered from a severe and prolonged attack of acute rheumatic polyarthritis; the hypertension was, however, relatively slight,—only on two occasions did the diastolic level reach 90 mm. of mercury. The intravenous pyelogram revealed a horse-shoe kidney with the ureters given off from the lower part of the pelvis, thus permitting good drainage; there was no evidence of obstruction to the outflow of urine. The question naturally arises whether in this instance the horse-shoe kidney is the cause of the hypertension. It seems to us improbable that the horse-shoe kidney[6] was in any way responsible for the hypertension. Indeed, if the congenital malformation in this instance is the explanation of the hypertension, it is the exceptional case in this series.

The common factor in all of these children was the rheumatic infection. All had suffered from severe prolonged attacks of acute

[6] Gutierrez (18), who has made the most extensive study of horse-shoe kidneys, states that there are three groups—the first, in which the horse-shoe kidney is asymptomatic and discovered accidentally at operation, by x-ray, or at the autopsy table; the second, in which there is no evidence of infection or of an abnormal renal function but in which there are signs and symptoms suggestive of the clinical syndrome of horse-shoe kidney disease; and third, in which there is serious renal disease. In discussing the clinical manifestations of horse-shoe kidney Gutierrez points out that the isthmus of the kidney lies in front of the aorta and the major symptoms are those produced by pressure upon the aorta and the solar plexus. The symptoms which he mentions are abdominal pain, constipation, and pain, edema and cyanosis of the lower extremities. He also mentions myocardial fibrosis and aneurysm of the aorta, which he believes to be secondary to the strain placed upon the heart by the pressure on the aorta; this, however, is open to question as the patient in whom an aneurysm developed also suffered from syphilis. Nevertheless, the main symptoms which he describes are those resulting from pressure upon the abdominal aorta which would cause a hypertension in the upper extremities. The fact that in our case the blood pressure in the lower extremities was higher than in the upper extremities clearly excludes this as an explanation of the hypertension. Furthermore, there was no evidence of serious renal disease. Therefore, it would seem that our case belongs in the first group, *i.e.*, with those which are asymptomatic and discovered accidentally.

rheumatic fever. For this reason we believe that the hypertension observed in these cases is in all probability in some way related to an active rheumatic infection.

GROUP II. PATIENTS SUFFERING FROM RHEUMATIC INFECTIONS WHO HAVE SHOWN REPEATED ELEVATIONS OF THE BLOOD PRESSURE BUT IN WHOM THE EXACT SIGNIFICANCE OF THE HYPERTENSION IS NOT CLEAR

There remains for consideration a group of children in whom during the course of their illness repeated high blood pressures have been recorded over a period of a year or more. The majority of cases still show wide fluctuations in the blood pressure which make it difficult to evaluate the significance of the hypertension. In view, however, of the children who have developed essential hypertension we believe that these high blood pressures warrant investigation.

The group is composed of 12 children, all of whom have suffered from repeated or prolonged attacks of acute rheumatic fever or chorea. All have shown high diastolic pressures on several occasions. Four first showed hypertension at 10 years of age; 1 at 11; 2 at 12; and 4 at 13. The majority still have blood pressures which fluctuate between a normal level and a hypertensive level. Some of the children (Cases 29, 43 and 44) were of an excitable temperament which rendered it difficult to evaluate the blood pressure. In the majority of these cases there was some evidence of a slight but persistent active infection as indicated by an acceleration of the sedimentation rate, a prolongation of the conduction time, a persistent fever or tachycardia, or the development of murmurs. Although several of them have shown a relatively persistent tachycardia, in no case has there been a definite correlation between the rapidity of the pulse and the height of the blood pressure. The synopses of the case histories (given in the appendix) indicate the relation of the hypertension to the rheumatic illness.

The usual causes[7] of hypertension were all excluded. The results of the renal investigations, which are given in Table V, show that the kidney function in each instance was normal. In only two cases did

[7] See *Criteria and Selection of Cases.*

TABLE V

Borderline essential hypertension

CASE NO.	NAME	AGE	SEX	COLOR	RHEUMATIC HISTORY	HYPERTENSION 1ST OBSERVED		STATUS IN 1936				
						Age	B.P.°	B.P. & Ht.	Urine	N.P.N.	P.S.P.	Pyelogram
38	K. Q. 85045	11	F	W	Chorea 1st att. 8 yrs. Rh.M., M.I. 8½ yrs. Ch.Rh.M. 9–11 yrs. P. 108–140. B.M.R. + 2	10	130/95	130/80 Ht. norm. Sys. at apex ?	1.028 Alb. 0 Micro.: neg.	32	50% ½ hr. 65% 1 hr.	Normal
39	H. P. 56907	13	M	W	Rh.H.D. c̄ M.I. 10 yrs. P. 110–135 B.M.R. + 3	10	125/90	125/88 M.I.	1.012 Alb. 0 Micro.: neg.	34	25% ½ hr. 65% 1 hr.	Normal
40	A. K. 86940	13⅓	F	W	Rh.F. 1st att. 5 yrs. 2nd att. 11 yrs. 3rd att. 12 yrs. M.S. 13 yrs.	13	130/90	125/75 M.I. & M.S.	1.020 Alb. tr.	34	50% ½ hr. 76% 1 hr.	Normal
41	B. W. 54003	16	F	W	No ac. rh. illness M.I. 12 yrs. M.S. devel. between 15 & 17 yrs.	13¾	130/90	120/90 M.I. & M.S.	1.020 Alb. 0 Micro.: Ep.C.	37	30% ½ hr. 65% 1 hr.	Normal
42	H. L. 44688	14	F	W	Chorea 1st att. 10 yrs. 2nd att. 11½ yrs. 3rd att. 12 yrs. Rh.M., M.I. 12 yrs.	12½	135/95	140/90 M.I. P-R 0.22 S.R. ++	1.018 Alb. +	37	40% ½ hr. 70% 1 hr.	Only one kidney visualized
43	J. K. 80845	15	F	W	Chorea & Rh.M. 12 yrs. Choreiform movements 12–14 yrs.	13	140/90	120/80 Ht. norm. in size M.I.	1.030 Alb. 0 Micro.: Ep.C.	39	37% ½ hr. 75% 1 hr.	Normal

44	V. L. 38309	15	F	W	Rh.H.D. 11 yrs. Chorea 12 yrs. Rh.M. c̄ P. 100 12–14 yrs.	13	130/95	130/80 Ht. norm. Sys. at base	1.024 Alb. + Micro.: Occ. W.b.c. Ep.C. ++	35	30% ½ hr. 75% 1 hr.	Normal
45	J. C. 65294	14½	M	W	Rh.F. 1st att. 8 yrs. 2nd att. 10 yrs.	10	115/90	130/85 M.I., sl.	1.022 Alb. + Occ. casts	37	65% ½ hr. 70% 1 hr.	Normal
46	F. C. 67504	16	M	W	Chorea 10 yrs. 2nd att. 11 yrs	12	130/90	138/92 Ht. norm.	1.022 Alb. 0	35	45% ½ hr. 70% 1 hr.	Normal
47	A. R. 58699	18	F	W	Rh.F. 9 yrs. Chorea 10 yrs. Rh.M. 10 yrs.	10½	140/90	140/88 M.I.	1.010† Alb. 0	29	25% ½ hr. 60% 1 hr.	Normal ? One pelvis dilated ?‡
48	E. H. 59464	15	F	W	Chorea, 10 attacks 1st att. 6 yrs. 10th att. 12 yrs. Rh.M. 11 & 12 yrs.	11	135/100	128/90 Ht. norm.	1.022 Alb. tr. Micro.: Ep.C. ++			
49	S. C. 65234	14	F	W	Chorea, 4 attacks 1st att. 8 yrs. 4th att. 14 yrs.	14	130/90	130/100 Ht. norm.	1.017 Alb. 0 Not rechecked since 1932	36	60% 1 hr.	

* Highest reading.
† Voided specimen.
‡ Kidney pelvis considered normal by roentgenologists and urologists.

the pyelogram reveal any deviation from the normal[8]. In neither instance did the abnormality seem to us of significance. None of the children showed any evidence of arteriolosclerosis. All had negative blood Wassermann reactions.

The common factor in all the cases is the rheumatic infection,—chorea or rheumatic fever or both. Neither the importance of this factor or the ultimate significance of the repeated high level of the blood pressure can be determined at the present time. One girl (No. 49) appears to have essential hypertension but, as she was not seen prior to her fourth attack of chorea and has always shown hypertension, it is impossible to determine whether the hypertension is in any way related to the repeated attacks of chorea. In the other cases it remains for the future to reveal whether the blood pressure will stabilize at a normal level or whether they, like the cases just reported, will develop essential hypertension. The solution of this problem will require years of observation.

DISCUSSION

Hypertension has been observed in 49 children suffering from chorea, chorea and rheumatic fever, or rheumatic fever alone. Seven cases of hypertension have been observed in children suffering from chorea; 14 in children suffering from chorea and acute rheumatic fever; and 28 in children suffering from rheumatic fever. This gives a ratio of 1 to 2 to 4. In our clinic the incidence of these various rheumatic manifestations is 1 to 3.5 to 6. The number of cases of hypertension which have been observed is too small to be of any statistical value. The figures merely indicate that the phenomenon occurs in all three types of rheumatic infection. In our small group approximately one-half of the cases occurred in boys and one-half in girls. Forty-one were in the white race and 8 in the colored. The numerical

[8] In Case 42 only one kidney was visualized. It so happened that, contrary to the usual practice, only one x-ray plate was taken. Unfortunately, the patient moved to Virginia the following morning thereby rendering further investigation impossible. Therefore, no conclusions can be drawn concerning the status of the other kidney. In the other case (No. 47) the pelvis of the right kidney appeared to be slightly dilated; however, both the roentgenologists and the urologists considered it to be within normal limits.

preponderance of white to colored is not as significant as it may appear to be at first glance because the proportion of the two races in our clinic is 2.3 white to 1 colored. As regards the age of incidence, 12 children were under 10 years of age; 16 were between 10 and 12 years; and 24 were 12 years or older when hypertension was first observed. In brief, hypertension has been observed in both sexes, in both the white and the colored race, and at all ages at which rheumatic fever is common.

The fact that the majority of cases of hypertension were observed in children of 10 years or older raises the question of the effect of puberty upon the blood pressure. There is a general belief that the elevations of blood pressure occurring at the time of puberty are physiological rather than pathological and this phenomenon has been called a "hypertension of puberty" (1).

Our recent experience in the cases which have developed essential hypertension demonstrates that there is a wide fluctuation in the early stage of essential hypertension and that the finding of a normal blood pressure after a period of hypertension does not prove that the high readings were of no serious import. In other words, a hypertension which occurs at puberty cannot be considered to be insignificant until such cases have been followed over a period of years.

This point of view raises the question of what constitutes a normal blood pressure at the time of puberty. A review of the literature shows a wide variation in the normal standards for children. The findings of various authors are given in the chart in Figure 2[9]. In each instance the mean blood pressure at a given age is recorded. The curves show a variation of 10 mm. in the mean diastolic level and of 20 mm. in the mean systolic level. It must be also noted that a difference of opinion exists concerning the extent to which the blood pressure can deviate from the norm before it should be considered abnormal. Most investigators believe the standard deviation to be 6 to 10 mm., over twice that amount to be regarded with suspicion, while a threefold increase is almost certainly pathological.

All investigators agree that the widest variation in the blood pressure

[9] Faber and James, Burlage, and Richey read the diastolic at the fourth phase. Meyers' is a composite chart for a number of investigators. Stocks and Karn record the diastolic level at the fifth phase.

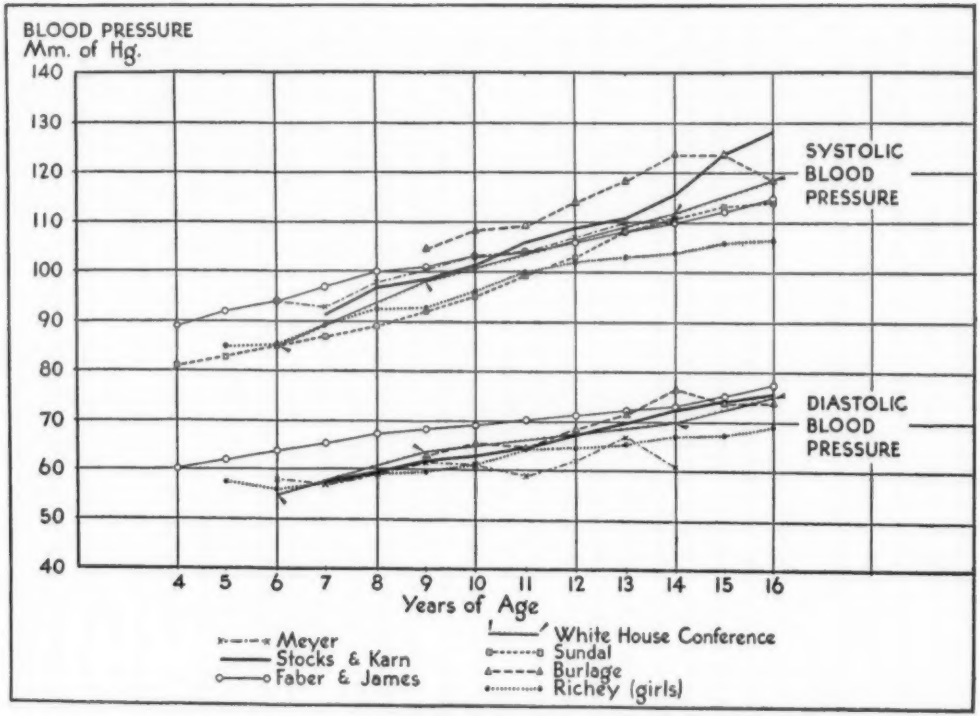

FIG. 2. COMPOSITE CHART SHOWING BLOOD PRESSURE IN RELATION TO AGE, AS DETERMINED BY VARIOUS INVESTIGATORS

occurs at the time of puberty. Faber and James (12) obtained a standard deviation of 5.5 to 7.5 mm. in children up to 14 years. Applying these standards to their figures it will be seen that a diastolic pressure of 90 mm. Hg., if not clearly abnormal, is always to be viewed with suspicion. Richey (26) in his study of over 1000 children reported only three girls and five boys with a diastolic blood pressure over 89 mm. Hg. At the White House Conference (35) no attempt was made to define the precise limits of normal blood pressure in children; instead, the report published by the Conference gives a few average figures and states that a variation of ± 10 mm. is "roughly within normal limits." Stocks and Karn (30) in their investigation found that the standard deviation for the systolic level varied between 9 and 14 mm. and that for the diastolic level varied between 7 and 11 mm. Hg. They do, however, state that a diastolic over 90 is "almost certainly pathological" for a boy of 10 years. On the other hand, in the case of a boy of 16 years they become suspicious only when the blood pressure reaches 151/95, and they do not consider it clearly pathological until it reaches 163/104.

In contrast to this, Sundal (31) in his extensive study of blood pressure between the ages of 3 and 20 years views with suspicion a systolic pressure of 130 mm. Hg. These findings are in harmony with those of other investigators who have made a study of the normal blood pressure of the young adult, 15 to 20 years of age. Thus Alvarez (2,3), in his extensive researches among young adults, considers 127 mm. a high normal systolic level for women and 130 mm. the upper limit of normal for young men (16 to 40 years of age). Figures given by various life insurance companies (13, 14, 21, 27, 28, 29, 32) indicate that between 15 and 19 years of age the average systolic range lies between 119 and 136 mm. Hg., the average diastolic is below 80 mm. and 80 to 85 mm. is high normal diastolic. Ayman (5, 6) in his study of essential hypertension considers 150/90 as evidence of hypertension at 50 years of age and believes that a blood pressure above 140/80 between the ages of 14 and 29 years should be viewed with suspicion. From these reports it is clear that the normal range given by Stocks and Karn is far higher than that given by other investigators.

Additional evidence that these blood pressure readings are abnormally high is shown by the results which were obtained by one of us (M.S.H.) on a small group of normal school children.[10]

Five hundred and seventy-five blood pressure determinations were made on a group of 110 normal children of whom 47 were colored and 63 were white. The average blood pressures for the entire group were 97/67 at 8 years, 104/70 at 10 years, 107/72 at 12 years and 110/73 at 14 years. In comparison with the blood pressures obtained by other observers, the average blood pressures are slightly higher, but they are clearly within the limits of the normal variation. Inasmuch as the present paper is concerned not with *average* blood pressures but with the occurrence of hypertension, the maximum isolated systolic and diastolic blood pressure readings are of interest.

The *maximum systolic blood pressure* recorded was 134 in a colored girl of 12 years whose diastolic pressure was 76 mm. Of the total group only ten children had a systolic blood pressure over 120 mm. Of these, one child of 8 years had single reading of 126/60 and one child of 10 years had a blood pressure of 132/80 on one occasion. The remaining eight children were 12 to 14 years of age. Four of these children had a systolic level over 130; the two highest individual readings were 134/76 at 12 years of age and 132/80 at 14 years of age.

As regards the *maximum diastolic pressures*, there was only a single isolated diastolic reading of 90 mm. (*i.e.*, one pressure of 120/90) observed in a colored girl of 12 years of age. There were in addition in seven individuals eight other single diastolic readings over 85 mm. of mercury. One child of 8 years had a blood pressure of 120/86. The remaining six were between 11 and 13 years of age; the highest blood pressure in this group was 118/88. These figures, although not conclusive, offer further evidence that the blood pressure findings in cases of rheumatic fever and chorea are abnormally high.

[10] Between November 1936 and May 1937 a white orphanage and a colored school were visited on alternate weeks and blood pressure readings were taken on two groups of children, one 6 to 10 years of age and the other 11 to 15 years of age, at each institution. The determinations were made on each group at monthly intervals, but owing to absences not every child was present every time. Therefore, in some cases only four blood pressure readings were recorded and in others there were five or six separate determinations.

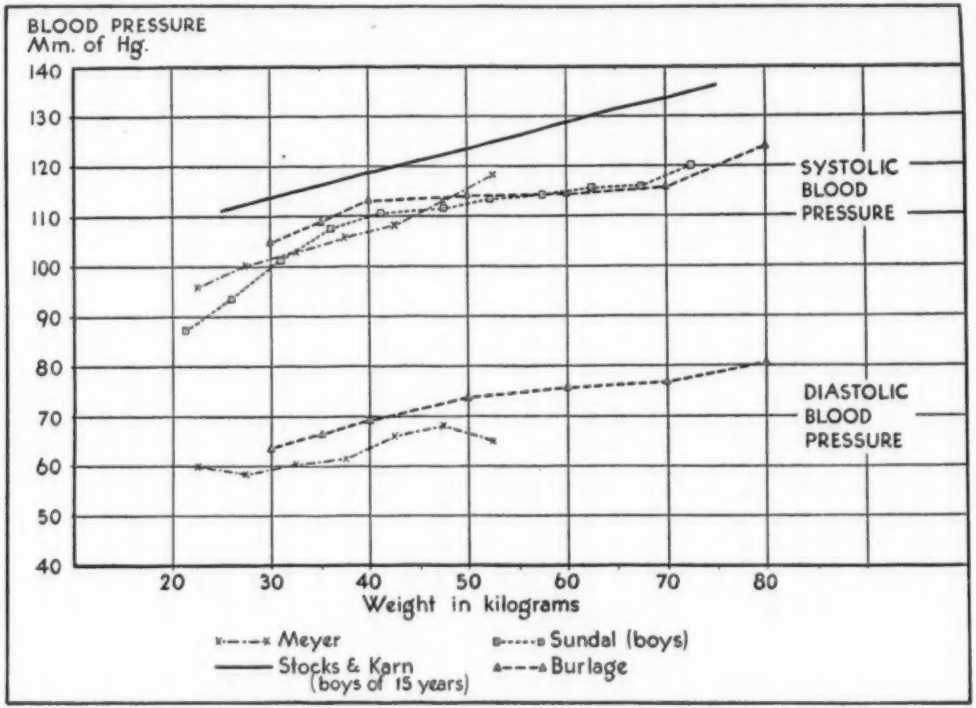

FIG. 3. COMPOSITE CHART SHOWING BLOOD PRESSURE IN RELATION TO WEIGHT IN KILOGRAMS

The next factor to be considered in connection with the hypertension is obesity. Various investigators claim that there is a closer correlation of blood pressure with weight than with age. The results of the most comprehensive studies are shown in Figure 3. The figures charted are those of the highest average blood pressure for a given weight. A comparison of these curves with the curves correlating blood pressure with age shows that the norm stands at a slightly lower level. The heaviest child in our study (Case 49) weighed 142 pounds at 15 years and was 62 inches in height; the blood pressure varied between 130/100 and 164/102. Comparing our figures with all those preceding, we find that our blood pressures when correlated with weight are quite as abnormal as when correlated with age. Therefore it is clear from all these studies, whatever standards are used, that, after due allowance is made for puberty and obesity, the high blood pressures found in our investigation must be viewed with suspicion.

Finally, the question may be asked whether the elevation of blood pressure was merely indicative of a poor response of the cardiovascular system to exercise. At first glance this explanation might seem plausible because in a large number of cases—approximately two-thirds—the elevation of blood pressure occurred at a time when the child was up. Most investigators (7, 10, 15, 36) agree that the response of the blood pressure to exercise closely parallels that of the pulse rate; and by the time the pulse rate has returned to normal the blood pressure too has returned to the pre-exercise level. Inasmuch as all our blood pressure determinations were made when the children were quiet and pulse rates basal, it does not seem probable that the elevation of blood pressure represents a poor response of the cardiovascular system to exercise. Indeed, if exertion is a factor, in our judgment the reverse statement would be more tenable, namely, that bed rest tends to keep hypertension in abeyance.

In view of all the evidence, our findings are indicative of true hypertension. The exact relation of the hypertension to rheumatic infection is, however, not clear. It may be a specific response to the rheumatic infection; it may be that there are a number of infections which are associated with an elevation of the blood pressure, of which rheumatic fever is one. It remains true that the frequent occurrence of hypertension in close connection with an acute rheumatic episode suggests

that the hypertension in these cases is probably in some way related to, or exaggerated by, the active rheumatic infection.

A survey of the literature yields little information concerning the occurrence of hypertension in children suffering from acute rheumatic fever. Hensen (19), in 1900, in a discussion of the variations in blood pressure in health and disease, reported four cases of recent rheumatic infection in which the systolic pressure was found to be elevated. Potain (25) in 1902 reported a similar finding in 11 out of 24 patients suffering from a mild, chronic rheumatic infection. These observations, however, have not been confirmed. Indeed, Meyer (22) in her recent report concerning blood pressure in health and disease states that in seven cases of acute rheumatic fever the initial blood pressure taken at the onset of the disease was low. Winkler (37) has reported the occurrence of hypertension in chorea. He states that the blood pressure tends to rise during the acute illness and that it returns to normal during convalescence. He also emphasizes the great lability of the blood pressure in this disease. In adults there are no reports concerning the occurrence of hypertension associated with *acute* rheumatic fever.

The frequent occurrence of hypertension with mitral stenosis has been reported by various observers. Thus many years ago Goodhart (16) (1880) and Pitts (24) (1887) commented on the frequent finding at autopsy of granular kidneys in cases with valvular heart disease. About twenty-five years later Cowan and Fleming (11) published a report on the association between mitral stenosis and renal fibrosis. In 1926 Boas and Fineberg (8) reviewed a large series of cases of chronic rheumatic heart disease and found that hypertension occurred in 29 per cent of the patients with mitral stenosis and in 27 per cent of those with aortic insufficiency who were over the age of 40. In 1928 Levine and Fulton (20) reviewed 762 cases of mitral stenosis and found that over 50 per cent of the patients over 45 years of age had hypertension. The authors emphasized the fact that their report was concerned with mitral stenosis, not necessarily of rheumatic origin. Furthermore, they found that the blood pressure in the younger patients with mitral stenosis (15 to 35 years of age) was below normal. In a recent paper Gupta (17) discussed not the phenomenon but the cause of the hypertension occurring in mitral stenosis. This,

he believes, is a nerve reflex caused by the small volume of blood expelled from the left ventricle into the aorta and in no sense caused by the rheumatic infection. Such an explanation would not be valid in our cases for in the majority of instances there was no evidence of mitral stenosis.

The fact that two children have developed essential hypertension (34) raises the question whether there is any relation between hypertension observed in the older patients with mitral stenosis and the hypertension we have observed in acute rheumatic fever. These children must be followed over a period of years before the diagnosis of essential hypertension can be excluded. It is, however, at the present time only a matter of speculation whether these children who show transitory periods of hypertension represent the group who in adult life will develop essential hypertension.

Although the majority of our cases were observed in children at the stage of puberty, it must be remembered that the same absolute value of 90 mm. of mercury was used for all children, regardless of age, as an indication of hypertension. The error introduced by the assumption of a uniform diastolic blood pressure for all ages is indicated in the norms given in Figure 2. These graphs show that the absolute value of 90 mm. is abnormally high for the younger age group. Therefore, we believe that hypertension occurs in young children (4 to 8 years of age) with greater frequency than our figures indicate.

These observations disclose another aspect of the problem which calls for investigation. *It may be that the rheumatic infection makes manifest an underlying inherent tendency to hypertension.* This possibility deserves serious consideration, inasmuch as all children with a rheumatic infection do not develop hypertension and furthermore essential hypertension (33) is known to occur in children who are free from rheumatic infection. The large number of instances of hypertension which we have observed in children suffering from rheumatic infections suggests the further possibility that people with inherent tendency to hypertension may be especially susceptible to acute rheumtic fever.

It must, however, be remembered that, although the determination of blood pressure has been a routine procedure in our Cardiac Clinic,

it is not a common procedure in pediatrics. Therefore, in childhood, both in health and disease, there is a great paucity of blood pressure determinations. For this reason we are unable to make any comparison between the frequency of hypertension in rheumatic fever and in other illnesses. The possibility exists that hypertension in childhood may be a far more frequent phenomenon than has heretofore been believed and that it has no specific relation with rheumatic fever. Regardless of the ultimate interpretation of the phenomenon, this study demonstrates the occurrence of hypertension in children with acute rheumatic fever and chorea. In addition, it illustrates that hypertension presents an important problem in childhood.

CONCLUSIONS

(1) Thirty-seven cases are reported in which hypertension occurred in close connection with an acute rheumatic episode—either chorea, chorea and rheumatic fever, or rheumatic fever.

(2) Twelve additional patients are reported in whom repeated high blood pressure readings have been recorded over a long period of time but in whom the blood pressure still fluctuates widely. These patients have not been followed for a sufficient length of time to determine whether the hypertension will become permanent.

(3) The hypertension in these cases was not associated with demonstrable renal damage.

(4) The various conditions under which hypertension has been observed are analyzed.

(5) The frequent association of hypertension with some manifestation of rheumatic activity strongly suggests that the hypertension in these cases is related to an active rheumatic infection.

(6) The exact relation between the hypertension and the rheumatic infection is not clear. Various possibilities are discussed.

APPENDIX TO GROUP II

Synopses of the Salient Features of the Histories Showing the Relation of the Hypertension to the Rheumatic Infection

Case 38. A white girl of 11 years who had her first attack of chorea at the age of 8. For the ensuing two years, 1933–35, she suffered from rheumatic myocarditis, showing fever, tachycardia, and a gallop rhythm on slight exertion. Throughout the spring of 1935, when just 10 years of age, the diastolic level of the

blood pressure was constantly at 90. Since then the blood pressure has fluctuated between 110/70 and 130/95. Because of the persistent tachycardia, it is difficult to evaluate the blood pressure. There has been, however, no correlation between the tachycardia and the periods of hypertension.

Case 39. A white boy of 13 years who has been ill a great deal. He is of an excitable temperament which renders it difficult to evaluate the blood pressure. He has never had a frank rheumatic episode, but in 1933 was found to have developed evidence of a rheumatic myocarditis as evidenced by a tachycardia, an apical systolic murmur which was transmitted to the axilla, and an electrocardiogram showing a P-R interval of 0.16 to 0.20 of a second; the sedimentation rate was accelerated. The blood pressure at that time was 125/90. Although the sedimentation rate returned to normal, the tachycardia persisted; there has, however, been no correlation between the tachycardia and the level of the blood pressure. The blood pressure has reached 130/90 when the pulse rate was 110 and it has been 120/80 with a pulse rate of 135.

Case 40. A white girl of 13½ years who had her first attack of acute rheumatic fever at 5 years and a second attack at 11 years. In 1934, at the age of 12, she had another attack of rheumatic fever. She was acutely ill from March to July and at a convalescent home until February 1935. Finally, in September 1935 she returned to school. In December 1935 her blood pressure first reached 130/90. Throughout the ensuing six months the blood pressure fluctuated between 115/70 and 130/90. During this time, although asymptomatic, she developed signs of mitral stenosis.

Case 41. A white girl of 16 years who in 1931, at 12 years of age, developed signs of a rheumatic myocarditis and a mitral insufficiency. During 1931 and 1932 she showed definite signs of an active rheumatic infection. Throughout 1933–34 the pulse rate remained accelerated (100 to 120) and the blood pressure, varied between 130/90 and 110/80. During this time, although asymptomatic, she developed signs of mitral stenosis. In 1936 the pulse slowed to 95 per minute, but the blood pressure remained 120/90.

Case 42. A white girl of 14 years who was first seen in the Harriet Lane Home at 12 years of age in December 1933 after her third attack of chorea. At this time she had rheumatic heart disease with mitral insufficiency and the P-R interval was 0.20 of a second. Blood pressure 135/90. She was kept in bed for many months and since then has been up quietly at home. Although she is now clinically well, she still shows a delayed conduction time and an accelerated sedimentation rate. The level of the blood pressure has continued to fluctuate widely and it has not infrequently reached 130/90.

Cases 43 and 44 are almost identical. Both children are white girls of 15 years who have suffered from chorea and rheumatic myocarditis. During the last two

years both have shown wide fluctuations in the blood pressure, and although both are of excitable temperament, there has been no correlation between the pulse rate and the blood pressure.

The next four children in this series (Cases 45, 46, 47 and 48) all appear to be entirely well. Three of them felt so well that they have returned to the clinic only at infrequent intervals. The other child is now under the care of a private physician. Inasmuch as our data are insufficient to determine whether these blood pressures are persistently elevated, we have placed these children in the group of hypertensive cases.

Case 49. A white girl of 14 years who has had four attacks of chorea. The blood pressure, when first recorded after her fourth attack of chorea, was 130/90. During the following year (1931–32) the blood pressure fluctuated between this level and 160/100. In the autumn of 1932 there was no evidence of impairment of renal function. In April 1936 the blood pressure was 130/100. Unfortunately we were not permitted to recheck the status of the kidneys.

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RESEARCHES ON TETANUS[1]

VII. (1) The Time Required for the Fixation of a Fatal Quantity of Tetanus Toxin; (2) The Return Passage of Toxin by Way of the Lymphatic Capillaries to the Cardiovascular System; (3) The Return Passage as the Basis of a Method for the Approximate Determination of the Volume of Lymph in the Closed Lymphatic System

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1. The Time Required for the Fixation of a Fatal Quantity of Tetanus Toxin

1. In Paper V, entitled "The Distribution and Fate of Tetanus Toxin in the Body," Abel, Evans and Hampil have given a preliminary account of the results of a number of experiments dealing with this problem. A section of that paper,[2] introductory to the experiments there given, is entitled "Further experiments to prove that the toxin is soon so firmly and irretrievably fixed by the susceptible and other tissues of the body that it can no longer be removed from a living animal by repeatedly infusing it with normal blood." Fixed toxin, as defined in that paper, signifies toxin that is so firmly bound that it is no longer recoverable by the use of normal blood or salines from the cells of an organ that has had time to absorb and bind it.

It has been proved, for example, that the spinal cord of an animal that has been injected intravenously, intramuscularly, or subcutane-

[1] A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of observations on tetanus.

[2] Page 369, *et seq.*

ously with many lethal doses of the toxin can no longer give up its fixed toxin—the only form in which toxin is present in it—to the blood of a control animal or to a physiological salt solution; nor will a ground-up emulsion of such a cord induce tetanus in guinea-pigs or mice that have been inoculated with it.[3] In other words, biological assays, made with a spinal cord containing fixed toxin only, give not the slightest indication of its presence. And this holds for all other organs upon which the toxin has been fixed, when parts of them are injected into test animals, provided such organs no longer contain toxiferous blood and lymph. Bio-assays can do no more than give us information in respect to the amount of unbound toxin that happens to be present in the cardiovascular and lymphatic systems. Experiments of the kind presently to be described induced us to say that it is only the fixed, non-recoverable and non-assayable fraction of the toxin that acts upon voluntary muscles and central neurons and causes death after a fatal dose has been administered.

It was furthermore pointed out in Paper V that when very susceptible animals, such as the sheep and the horse, have received several lethal doses of the toxin, the excess over the amount that has been fixed in the body continues to circulate in the blood vascular and lymphatic systems. It was also shown that the time of death is predetermined by the absolute amount of toxin that has been fixed by the specifically reacting organs of the body, in the first few hours during which their cells were exposed to the requisite threshold concentration of this poison.

It was the original object of the section entitled "Further experiments," etc., of Paper V, to determine how long a time must elapse after the intravenous injection of from 1½ to 3 and more lethal doses[4]

[3] See pages 366 and 367 of Paper V.

[4] We have found in the course of very numerous experiments extending over a period of five years that injection of 480 guinea-pig L.D. 50's per kilogram invariably induces fatal tetanus in dogs, of medium weight and age, and have therefore arbitrarily chosen this figure as our lethal dose for dogs. This dose kills dogs, taken at random, in from 5 to 9 days. Neither a median nor a minimal lethal dose, but certainly larger than a statistically determined median lethal dose would be, it was adopted as a matter of convenience. We have always stated the dosage of the injected toxin in terms of standard guinea-pig units, so that our work can readily be checked by others without reference to our arbitrary dog unit.

of the toxin into dogs, before their organs have absorbed and fixed enough of it so that death will inevitably follow. This point could be definitely established by removing, at the time when fixation had presumably been effected, the excess of the toxin that still circulated in the blood vascular and lymphatic systems. This removal was accomplished by the simple method of repeatedly bleeding and infusing the poisoned animals with normal blood.[5] Details of several of the experiments that were performed with this object in mind were given in condensed form in that section, and a promise there made to give, at a later time, a further account of this earlier work is now being fulfilled.

The protocols of the additional experiments that have been performed since Paper V was published fully corroborate the earlier statements given in that paper. But our additional experiments, as will be seen from protocols now to be given, have brought to light a new fact of theoretical importance, which serves to explain the limited efficacy of antitoxin treatment in human tetanus. It has been easy to prove by "wash-out" experiments that dogs whose tissues have fixed an amount of toxin, that inevitably leads to death when they are left to themselves, can be saved by an intravenous injection of a large excess of tetanus antitoxin, when the injection is made during the period of incubation that follows the fixation of the toxin. "Washed-out" control animals that have likewise fixed a lethal fraction of the injected toxin never survive.

2. *Brief statement of method of experimentation.* Dogs of medium weight that had recently attained maturity were selected for the experiments. The intravenously injected, highly toxic filtrates were well assayed in guinea-pig units. The exact time of the intravenous injection was noted, and after a certain time had elapsed the animals

[5] For various reasons sheep would have served our purpose better than dogs. The tissues of sheep bind no more than a full lethal dose of the toxin, even when as many as 10 such doses are injected, and it would have been of interest to learn whether the time required for the fixation of a fatal dose of the toxin by their tissues differed from that required by the tissues of dogs, under the same circumstances. Unfortunately, however, the low tolerance of the respiratory apparatus of sheep, in respect to anesthetics, precluded their employment in experiments of long duration.

were lightly etherized and subjected to the following procedures. The femoral artery and the femoral vein were cannulated. Suitable arrangements were then made for repeatedly bleeding the animals by the femoral artery to the danger point and for immediately infusing them with amounts of heparinized normal dog's blood equal to those that had been withdrawn from the artery. The amount of normal dog's blood at hand for repeated infusions was 2½ to 5 liters.

3. *Protocols.* In order that our paper may not be unduly lengthened we shall first give a condensed account of the results of six experiments, inclusive of controls. The seventh experiment, X-16, in which the largest amount of toxin was used and in which certain points were more clearly established, will be described in greater detail.

The first two experiments of the entire series, X-1 and X-2, were of a preliminary and orienting character and served merely to establish the method of procedure that was finally adopted. From dog X-1 blood was removed at the first bleeding to the point of asphyxia; because of a defect in the apparatus, normal dog's blood could not be run soon enough into the femoral vein and the animal therefore failed to survive. Dog X-2 was bled and infused with normal blood only three times, and the obtained results could not be utilized because of the unreliability of the bio-assays, which were made with an insufficient number of guinea-pigs.

Experiment X-3. In experiment X-3 the process of successively removing and infusing blood was repeated only five times, with the result that a fairly large fraction of a lethal dose (0.25 dog L.D.) was left in the blood stream. This dog showed slight but definite head symptoms of descending tetanus on the fifth postoperative day. No other manifestations of tetanus, however, appeared and the animal eventually made a complete recovery. The animal was injected intravenously with 13,200 guinea-pig L.D. 50's (3.02 dog L.D.'s) of toxin and was bled 4 minutes later. In the course of five successive bleedings and transfusions 12,608 L.D. 50's were recovered. Assay of the last sample of blood (removed 53 minutes after the injection of toxin) for tetanus toxin showed that 121 guinea-pig L.D. 50's per kilogram (0.25 dog L.D.) had been left in the blood stream of the animal. This remanent toxin, together with a small undetermined amount left in the lymph and another small fraction of a lethal dose that was bound by the tissues in the four minutes preceding the first bleeding, eventually led to some manifestations of general tetanus but, since the tetanic symptoms were mild and the animal survived, it is obvious that less than a lethal dose was bound.

We have found that when toxin is injected intravenously into dogs, not the slightest symptoms of descending tetanus will appear, in the majority of animals, until the injected amount approaches ½ to ¾ of a dog L.D., in which event recovery nearly always takes place. Thus the slight tetanic symptoms shown by dog X-3

were undoubtedly the result of the fixation of an amount of the injected toxin that was in the neighborhood of ½ to ¾ of a dog L.D. The indications are that at least ¼ of a dog L.D. was bound during the 4 minutes that intervened between the injection of the 3.02 L.D.'s and the first withdrawal of the toxiferous blood; during this time the tissues were exposed to the full brunt of the injected toxin. The control dog (J-17), of approximately the same weight, which had received one-half the dose of toxin (*i.e.*, 1.5 dog L.D.'s) that was injected into dog X-3, died of tetanus on the 7th day.

Experiment X-8. In experiment X-8[6] an approximately equal amount of toxin, namely, 15,500 guinea-pig units (2.96 dog L.D.'s) was injected intravenously into a dog weighing 10.9 kg.; but here the toxin was allowed to circulate in the body for a much longer time than in Experiment X-3. One hour and 35 minutes after the injection, blood was withdrawn from the femoral artery and an equal volume of normal heparinized blood infused into the femoral vein. This procedure, repeated seven times, brought about the removal of 9,068 guinea-pig L.D. 50's and left in the blood stream 1,090 guinea-pig L.D. 50's (0.21 dog L.D.). According to our calculations the amount of toxin fixed at the time of the last bleeding, 5 hours and 25 minutes after the injection of the toxin, was 4,250 guinea-pig L.D. 50's (0.81 dog L.D.). That the toxin fixed by the tissues of the animal, however, actually amounted to less than one lethal dose is proved by the fact that the animal survived. The first symptoms of a descending tetanus appeared on the 5th day after injection of the toxin. On the 7th day these were pronounced, but the animal appeared to have no difficulty in eating or drinking. Improvement in the condition began on the 10th day. That the tissues of this animal (X-8) were estimated to have fixed a full lethal dose, instead of somewhat less, is an error that lies within the range of the variability encountered in statistical determinations of the potency of tetanus toxin, as determined by bio-assays.

Samples of blood were removed 30 hours and 54 hours after the injection of toxin. Calculations based upon the toxin content of these two samples show that the entire circulating blood still harbored at this time 272 and 163 guinea-pig L.D. 50's, respectively, at the 30 and 54-hour intervals. Thus the fixation of the injected toxin was practically completed within a few hours after its intravenous injection.

Experiment X-10. In experiment X-10, a dog weighing 6.9 kg. was injected intravenously with 9,880 guinea-pig units of toxin (2.98 dog L.D.'s). Exactly 4 hours and 41 minutes after the injection of toxin, 340 cc. of blood were removed from the femoral artery. Immediately thereupon the animal was infused by way of the femoral vein with an equal volume of normal heparinized blood, under pressure of about three feet of blood, with an inflow period of three minutes. After allowing an interval of 6 to 10 minutes for the proper distribution of the

[6] Experiments X-8, X-10 and X-12 have already been presented briefly in Paper V. Several minor arithmetical errors, which were found in going over the calculations involved in these experiments, have been corrected.

infused blood the animal was bled again from the femoral artery, the same amount of blood as before being allowed to escape from the artery. Immediately, as before, a volume of normal heparinized blood equal to that removed by way of the artery, was introduced into the femoral vein. The operations of bleeding and infusion of normal blood, with duly recorded brief periods of rest, were repeated seven times in all. The dog passed through a five-day period of incubation without any sign of disturbed health, and showed the first beginnings of descending tetanus at the end of the fifth day. The symptoms of generalized tetanus increased in severity on the following days and became so severe by the 8th day that we believed the animal could not recover. It was certainly close to death, with muscular rigidity, inability to walk, pronounced trismus, profuse salivation and difficulty in swallowing water and finely chopped meat. These severe symptoms continued for two days (from the eighth to the tenth day), after which the animal began slowly to improve; it was gassed a month later, when it still showed the marked signs of emaciation always seen in severe cases of experimental tetanus. A control animal (X-11), of the same weight, that had received an equal amount of the toxin, manifested the first symptoms of tetanus after an incubation period of three days, and succumbed to the disease on the eighth day.

The blood withdrawn at each of the seven bleedings was kept in a separate flask and the amount of toxin contained in each was assayed in terms of guinea-pig units. Ten minutes after the last, or seventh, infusion of normal dog's blood, 30 cc. of the animal's blood was withdrawn and its toxin content determined. On the assumption that the blood of a dog constitutes 10 per cent of its body weight, our bio-assays showed that the total amount of toxin that remained in the blood stream after the seventh bleeding and the seventh infusion of normal blood amounted to only 147 guinea-pig units. When this small residue is compared with the 9,880 units originally injected, it will be seen that it alone would be entirely ineffective in inducing any symptoms of tetanus in a dog of the given weight, as we know from many previous experiments. Now, it is interesting to know that in the course of the seven bleedings, which required one hour and twenty-two minutes, we actually removed 6,750 guinea-pig units of toxin. If to this amount we add the 147 units of toxin left in the animal's blood and an equal number of units left in the lymph, after the seventh bleeding, we learn that we can account, at the end of the experiment, for 7,044 of the 9,800 guinea-pig units originally injected. On the day following the operation the toxin content of the dog's blood was practically at the same level as after the seventh and last infusion; from this time on this small amount of remanent toxin gradually disappeared until only a trace of it was detectable on the sixth day.

A simple calculation shows that in the entire course of our experiment, the dog's organized tissues had extracted from the blood, and fixed in a non-recoverable manner, about 2,840 of the 9,880 units of toxin previously injected; that is, 0.86 of our dog lethal dose. There are indications that the relatively insusceptible animals used in our experiments continued to fix some of the toxin during the time that was taken up in the seven bleedings and infusions, particularly during the early

period when the blood still contained relatively much toxin. Even the 147 units left in the blood at the close of the experiment were finally absorbed and fixed by the tissues. This last fraction, however, would undoubtedly have been removed by another three bleedings and infusions, to the point at which bio-assays fail.

Experiment X-12. In this experiment, which terminated fatally, a 10-kg. dog (X-12) received 13,960 guinea-pig units (2.9 dog L.D.'s) intravenously, and was allowed to run about for seven hours. It was then anesthetized, and 7 hours and 40 minutes after injection of the toxin subjected to a sharp bleeding from the femoral artery. Normal blood was then infused into the femoral vein and this procedure of depleting and transfusing the animal was repeated seven times in all. In this animal the amount of toxin that was still retained in the blood vascular system after the final infusion of blood, was only 159 guinea-pig units (0.03 dog L.D.). The total amount of toxin that was here so firmly bound in 7 hours and 40 minutes as to withstand repeated infusions with normal blood, was 2.33 dog L.D.'s, 80 per cent of the injected amount of toxin. The toxin content of the circulating blood remained practically unchanged for 24 hours, but in 48 hours it had dropped to 40 per cent. In 72 hours marked head symptoms of descending tetanus were observed, together with some stiffness of the extremities. The tetanic symptoms became progressively more severe and the animal succumbed to the disease in seven days. A control dog (X-13), of approximately the same weight, that had received an equivalent dose of toxin per kilogram, died of tetanus in five days.

The following experiments (X-14 and X-15) are of particular interest because they show that when much less than three dog lethal doses of toxin are injected, more than four or five hours are required for the fixation of a full lethal dose. Dog X-14, for example, received intravenously 7,600 guinea-pig units of toxin, the equivalent of 1.2 dog lethal doses. Five hours and 25 minutes thereafter the first withdrawal of arterial blood took place. In the course of six bleedings and infusions of normal blood, 2,707 guinea-pig units of toxin were removed from the animal's body. Only one hour and two minutes were consumed in these proceedings and individual bleedings were not as large as they should have been, nor was their number large enough to effect more complete removal of the free toxin of the blood and lymph. At the time of the last bleeding, therefore, 6 hours and 27 minutes after the injection of the toxin, 854 guinea-pig units of the toxin were still present in the blood vascular system, and it is fair to assume that an approximately equal amount of the toxin still remained in the lymphatic system. The toxin retained in the two systems at the conclusion of the "wash-out" procedures amounted therefore to 1,708 guinea-pig units—the equivalent of 0.28 dog L.D. On adding the 2,707 guinea-pig units recovered by bleeding to the 1,708 units that remained in the blood and lymph, and subtracting this sum from the 7,600 units that were originally injected into the dog, we find that only 3,185 units had been fixed in the body from the time the toxin was injected until the end of the experiment. This means that only 42 per cent of the originally injected 7,600 units were absorbed and fixed by the tissues of the animal during the six hours and

27 minutes of their exposure to the circulating toxin. Only a small fraction of this 42 per cent could have been absorbed, however, during the six bleedings and infusions when the blood was being rapidly depleted of toxin. Now, this fixed 42 per cent of the injected 7,600 units falls far short of a lethal dose, and we may be sure that in this early stage of the period of incubation the dog's tissues continued to fix the relatively large number of 1,708 guinea-pig units (0.28 dog L.D.) that still remained in the blood and lymph. When this remanent number of guinea-pig units is added to what had been originally fixed the sum total amounts to 0.78 dog L.D. This quantity, which represents 65 per cent of the injected 1.2 lethal doses was sufficient to cause the death of the animal on the ninth day. The lethal amount of toxin here fixed (376 guinea-pig units per kg.) is 22 per cent less than the 480 guinea-pig units per kg. that we have taken as our dog lethal dose. The animal was injected on May 10. On May 13, the first signs of descending tetanus appeared, as evidenced by erection of the ears. May 14, the brows were wrinkled. May 15, the head symptoms of descending tetanus were more pronounced. May 16, there was beginning stiffness of the extremities. May 17, the head symptoms of descending tetanus were very marked; salivation was profuse, and all of the extremities were moderately stiff, with toes spread. On May 19, death occurred. This experiment shows very clearly that when so small an amount as 1.2 lethal doses of toxin is injected into a dog and the animal is kept confined to a cage, a lethal dose that is somewhat smaller than our standard lethal dose, is nevertheless fixed in the body.

Experiment X-15, illustrating the prophylactic effect of injecting, at the end of the "wash-out" experiment, a large amount of tetanus antitoxin into a toxin-poisoned dog. The preceding experiment (X-14), in which but little more than one dog lethal dose had been used, was intended to serve as a control for the following experiment (X-15), the chief purpose of which was to learn whether antitetanic serum, when injected after the removal of the circulating toxin, could prevent the inevitable consequences of the fixation of a fatal quantity of toxin that is no longer recoverable. Dog X-15, weighing 13.6 kg., received intravenously 11,400 guinea-pig units (1.7 dog L.D.'s against the 1.2 received by X-14) and four hours thereafter 365 cc. of blood were removed by the femoral artery, whereupon an equal volume of heparinized blood was infused into the femoral vein. The operations of bleeding and transfusing were repeated, with brief rest periods, seven times. All of these procedures together consumed only 56 minutes—an interval that was all too brief, in view of our later experiments. The average of the seven withdrawals of blood was also too small, as it came to only 24 per cent of the animal's total blood volume.

At the time of the first bleeding, 7,348 guinea-pig units (or 64.4 per cent of the injected 11,400 units) were present in the circulating blood. In the course of the seven bleedings, a sum total of 6,285 guinea-pig units were removed. Assay of the toxin content of blood taken three minutes after the seventh, and last, infusion of normal blood showed that the blood vascular system still harbored 990 guinea-pig units of toxin, and we may safely assume that the same number of units was

also present in the entire lymphatic system. Therefore, a total of 1,980 guinea-pig units (or 0.3 dog L.D.) was still present in the two systems.

On subtracting the amount of toxin that was removed in the seven bleedings, plus what was left in the blood and lymph after the last bleeding, from the 11,400 units originally injected, we obtain the number of units that were fixed in the course of the entire experiment, that is to say, in the course of the four hours during which the injected toxin circulated in the body and during the 56 minutes consumed in the bleedings and infusions. The remainder is 3,135 guinea-pig units, which, when translated into dog units, signifies that the tissues of Dog X-15 had fixed 0.47 dog L.D. of the toxin during these intervals. This dog, whose tissues had been exposed to a somewhat larger amount of toxin than was the case with dog X-14, but for a much shorter period of time, fixed the same amount of toxin as did the latter animal.

Had nothing further been done to dog X-15 it would probably have died as did X-14, for the reason that the tissues, during the first stage of the incubation period, still had at their disposal, as did those of the other dog, at least 0.3 dog L.D. When the 0.47 dog L.D., that had been previously fixed, is added to this 0.3 dog L.D., that was subsequently absorbed and fixed, we have an effective total of 0.77 dog L.D.— an amount that practically equals the 0.78 dog L.D. that finally killed Dog X-14.

Instead, however, of leaving dog X-15 to its fate, we injected it intravenously, immediately after the last bleeding, with a relatively large dose of antitetanic serum and found that it survived without showing any manifestations of tetanus. As was stated above, control dog X-14 did not receive antitoxin and died, therefore, from the effects of the fixed 0.78 of a lethal dose. The fact that antitetanic serum when given during the period of incubation saved the life of dog X-15 led us to believe that antitoxin can reach and neutralize bound toxin and save animals whose tissues have fixed a lethal amount of this poison. The ability of tetanus antitoxin to unite with fixed toxin and save life during the incubation period is conclusively shown in the following experiment (X-16) in which several lethal doses of toxin were disposed of, in a manner that defied detection and all attempts to wash it out of the body. This experiment, and its controls, will now be described in detail, since in it certain important points were more clearly established than in those described above.

DETAILED DESCRIPTION OF THE EXPERIMENTAL PROCEDURE AS ILLUSTRATED IN EXPERIMENT X-16

The toxin employed was that present in toxic filtrate 31a which, according to a recently made preliminary assay, contained more than 50,000 guinea-pig median lethal doses per cubic centimeter. As stated elsewhere, we have found it advisable to re-assay our toxin each time it is used in an experiment of this type. Accordingly, appropriate dilutions of the toxin were injected into a number of guinea-

pigs on the morning of October 15. Calculations made five days later, in conformity with the practice described in Paper V, showed that filtrate 31a assayed 82,000 guinea-pig median lethal doses per cubic centimeter on the day the experiment was made, October 15, 1937.

Two healthy, adult dogs were selected:

X-16. Male, black and tan mongrel, about 2 years old. Weight, 9.54 kg.

X-17 (control). Male, brown and white mongrel, about 2½ years old. Weight 8.64 kg.

1.84 cc. of toxic filtrate 31a was mixed with 6.16 cc. of sterile broth and 4 cc. of the mixture injected intravenously into dog X-16 at 9:19 A.M.

3.62 cc. of the mixture was similarly injected into dog X-17 at 9:25 A.M.

Thus each of the two animals received approximately 7,900 guinea-pig units per kilogram, or a total of 16.5 dog L.D.'s. The two animals were kept in separate cages until we were ready to etherize them, in order that muscular activity might remain approximately the same in both.[7]

We next proceeded to collect, with sterile precautions, approximately 4 liters of normal dog's blood. This was done by cannulating one of the femoral arteries of each of four large dogs, under ether anesthesia, and bleeding them to death, the blood being collected in sterile flasks containing, as anti-coagulant, heparin dissolved in normal salt solution (250 mg. of heparin per liter of blood). The flasks of heparinized blood were kept at body temperature in a warm water bath.

Dog X-16 was given a subcutaneous injection of 16 mg. of morphine sulphate at 3:50 P.M., and etherization by intratracheal insufflation was begun at 4:10 P.M. We have for several years employed this method of administering ether, because of the ease and safety with which prolonged light anesthesia can thereby be maintained.

The left groin was shaved, washed with ether and painted with iodine and alcohol.

The operation, begun at 4:33, was conducted in accordance with well known principles of aseptic technique. The femoral artery and vein were exposed and freed from the surrounding connective tissue. The apparatus, all of which had been sterilized, was then assembled. Two graduated cylindrical vessels, known as Kelly bottles or flasks, were used, one for measuring the outflow from the femoral artery, the other for measuring the inflow of heparinized normal blood. The inflow flask was held in place on the padded rings of a heavy stand and its outlet was connected by a rubber tube with the venous cannula.

In order to forestall clotting, heparin solution was run back and forth through the tubing and cannulae, whereupon the cannulae were introduced into the femoral artery and vein.

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[7] See Paper I, page 125, for a discussion of the effect of muscular activity on the incubation period of tetanus.

	gr.
First removal of arterial blood, 5:10 to 5:13 P.M.	443
This first sample of arterial blood was drawn off into a separate flask. To it, as also to each of the following samples of arterial blood, 10 cc. of 10 per cent sodium citrate solution was added.	
Infusion of normal heparinized blood, 5:13:5 to 5:16:5	450
The infused blood was allowed to mix in the body for 6 minutes before the next bleeding was begun, and a like rest period followed each of the subsequent infusions of normal blood.	
Second removal of arterial blood, 5:22:5 to 5:25	443
Infusion of normal heparinized blood, 5:25:5 to 5:28	440
Third removal of arterial blood, 5:36 to 5:39	418
Infusion of normal heparinized blood, 5:39 to 5:41	420
Fourth removal of arterial blood, 5:47 to 5:50	350
Infusion of normal heparinized blood, 5:50 to 5:53	350
Fifth removal of arterial blood, 5:59 to 6:02	418
Infusion of normal heparinized blood, 6:02 to 6:06	430
Sixth removal of arterial blood, 6:16 to 6:20	418
Infusion of normal heparinized blood, 6:20 to 6:24	420
Seventh removal of arterial blood, 6:30 to 6:34:5	368
Infusion of normal heparinized blood, 6:34:5 to 6:37:5	370
Eighth removal of arterial blood, 6:44 to 6:47	368
Infusion of normal heparinized blood, 6:47 to 6:51	370
Ninth removal of arterial blood, 6:57 to 7:02	368
Infusion of normal heparinized blood, 7:02 to 7:06	375
Tenth removal of arterial blood, 7:13 to 7:21	418
The flow of blood nearly ceased for several minutes, but was restored after stripping of the tube dislodged a clot.	
Infusion of normal heparinized blood, 7:21 to 7:27	480

The blood removed in the second to the tenth bleeding was collected in a single large flask. At 7:37, ten minutes after the last infusion of normal blood, a final sample of 110 cc. of arterial blood was allowed to run into a separate flask for assay, to determine how much toxin was left in the circulating blood. At 7:45, ten hours and eighteen minutes after intravenous injection of 16.5 dog L.D.'s of the toxin, 38.5 cc. of antitetanic serum,[8] assaying 1,700 U.S. units per cubic

[8] For so generously contributing the large amounts of concentrated antitoxin used in this and in many other experiments that will be described in a later paper, we are greatly indebted to Dr. William A. Feirer, Medical Director of the Sharp and Dohme Laboratories, and to Dr. John T. Anderson, Medical Director of the Biological Laboratories of E. R. Squibb & Sons.

centimeter, was infused into the femoral vein, together with 100 cc. of blood. The femoral artery and vein were tied off and the cannulae removed. The wound was closed with interrupted silk sutures; the operation was completed at 8 P.M.

The animal was in good condition when removed to its cage.

At the conclusion of the experiment there were three flasks of blood to be assayed for toxin:

Flask 1, containing 443 cc. of blood, removed in the first bleeding, 7 hours and 41 minutes after injection of toxin

Flask 2, containing 3,569 cc. of blood, removed in the second to the tenth bleeding, inclusive

Flask 3, containing 110 cc. of blood, removed 8 minutes before the injection of antitoxin and 10 hours and 16 minutes after the injection of toxin

Injection of guinea-pigs with graduated doses of the above bloods was completed at 11 P.M.[9] The results of the assays were as follows:

Flask 1: Number of cc. containing 1 guinea-pig median lethal dose (guinea-pig L.D. 50) = 0.045

Toxin in flask 1 = 443/0.045 = 9,850 guinea-pig L.D. 50's

Flask 2: Number of cc. containing 1 guinea-pig L.D. 50 = 0.12

Toxin in flask 2 = 3,569/0.12 = 29,740 guinea-pig L.D. 50's

Flask 3: Number of cc. containing 1 guinea-pig L.D. 50 = 2.0

Toxin in flask 3 = 110/2.0 = 55 guinea-pig L.D. 50's

Toxin left in blood stream:

954/2 − 55 = 422 guinea-pig L.D. 50's, or 44 guinea-pig L.D. 50's per kg. (0.09 dog L.D.)

Toxin bound at the time of last bleeding, 10 hours and 16 minutes after injection of 16.5 dog L.D.'s:

Toxin injected minus (toxin washed out + toxin left in circulating blood and lymph at end of experiment) = 75,440 minus (9,850 + 29,740 + 55 + 844) = 34,951 guinea-pig L.D. 50's = 7.6 dog L.D.'s, or 46.4 per cent of toxin injected.

It is assumed that, at the time of the last bleeding, the lymphatic system also still harbored 422 units of the toxin—the number that was shown to be present in the cardiovascular system at this time.

When examined the following morning the animal was found to be somewhat listless, but was otherwise in good condition. After another twenty-four hours it returned to its normal lively state and soon began to gain weight. It was killed by another dog seven weeks later.

No manifestations of tetanus appeared at any time. It cannot be doubted therefore that this dog, whose tissues had disposed of 7.6 lethal doses of toxin in a manner that evaded detection by bio-assays, was saved in consequence of the prophylactic action of the injected antitoxin, even though it be assumed that the entire 7.6 lethal doses were fixed and that no fraction of this amount was destroyed.

[9] See Paper V for method of assay of tetanus toxin.

The control dog, X-17, which had also been injected intravenously with 16.5 dog L.D.'s of toxin, but which had not been deprived of circulating toxin by repeated bleeding and infusion of normal blood, exhibited symptoms of a fulminating tetanus 48 hours later. In addition to widespread muscular rigidity, as evidenced by wrinkling of the brow, erection of the ears, marked trismus and opisthotonos, there were frequent and severe tetanic convulsions. Death occurred approximately 72 hours after the injection of toxin. At autopsy no significant gross pathological changes were found.

A second control dog (X-18) received 16.8 dog L.D.'s and after being left undisturbed for ten hours and twenty minutes, was injected intravenously with 38.5 cc. of antitetanic serum, assaying 1,700 units per cc. At no time did this animal, which was observed carefully over a period of fifteen days, show the slightest symptoms of tetanus.

COMMENTS ON THE FOREGOING EXPERIMENTS

Fixation of tetanus toxin and the dependence of the degree of fixation upon dosage and the time factor. It can be demonstrated with the help of the "wash-out" method that a rapid fixation of tetanus toxin takes place in the body of tetanized animals. The method of plasmaphaeresis might perhaps have been equally serviceable for our purpose. By repeatedly removing large amounts of the blood from toxin-injected dogs and regularly replacing the withdrawn toxiferous blood with normal dog's blood we arrived at a stage, as shown in experiments X-12 and X-16, when all of the as yet non-fixed but still assayable free toxin present in the animal's blood and lymph had been abstracted, with the exception of an insignificant and immaterial residue. We then had animals in hand whose tissues had fixed a lethal, or more than lethal fraction of the injected toxin. Such animals are well adapted for the study of the action of fixed toxin in respect to its incubation period and the time of onset of the first symptoms of tetanus, and particularly in respect to the action of timely injections of antitetanic serum on neutralizing the toxin at its fixation-points and thereby saving life.

When three or more lethal doses were injected intravenously, the irreversible fixation of a lethal dose was completed in the relatively short time of five to seven hours. In experiment X-10, in which the dog's tissues were calculated to have fixed 0.9 of a lethal dose, out of the 3 lethal doses injected, it turned out that the animal barely survived the effects of a severe descending tetanus. Dog X-8, that had

received 2.96 (that is to say, 3) dog lethal doses, in which the time interval between the injection of this amount of toxin and the first bleeding was limited to one hour and 35 minutes, in which the number of bleedings was limited to seven and an average amount of 35 per cent of the total blood was withdrawn at each bleeding, a descending tetanus of noticeable degree occurred, but the animal nevertheless survived. In spite of the short waiting period of one hour and 35 minutes that occurred in this experiment the animal fixed, out of the three lethal doses injected, as much as 0.86 of a lethal dose, only a small fraction of which can be credited to any fixation that took place during the bleedings, since the amount of the circulating toxin was being rapidly decreased.

When so little as 1.2 and 1.7 dog lethal doses are injected, in place of 3 employed in the experiments just cited, the time that intervenes between the injection of the toxin and the first of the series of bleedings must be lengthened, in order to give the receptive tissues more time to absorb an additional fraction of that part of the toxin which always continues to circulate in the blood and lymph of the dog. Dog X-14 illustrates this point. This dog received only 1.2 lethal doses of the toxin, as determined by guinea-pig assays. Five hours and 25 minutes thereafter the first of the six withdrawals of blood took place. It has already been stated in the protocol dealing with this dog that only one hour and 2 minutes was consumed in the bleedings and infusions of normal blood. On averaging the volumes of blood withdrawn in the six bleedings it turned out that only 22.6 per cent of the total blood volume was withdrawn at each bleeding. This compares very unfavorably with similar values of the bleedings in other experiments. For example, in dog X-3 the average amount of blood withdrawn in each of the five bleedings was 41 per cent of the total blood volume, in X-10 the average in seven bleedings was 41 per cent, in X-12 it was 39.2 per cent, and in X-16 as much as 42.2 per cent.

It cannot be doubted that only an insignificant fraction of the 1.2 lethal doses received by dog X-14 would have remained in its blood and lymph at the end of the experiment, had we allowed the injected toxin to circulate for a few more hours before the first bleeding, and had we furthermore subjected the animal to ten sharp instead of seven smaller bleedings. In that event the death of the animal would have been

caused solely by the toxin that was fixed in the longer waiting period. As it was, the amount left in the blood at the end of the bleedings was 854 guinea-pig units out of a total injected number of 7,600 units. This remanent toxin is equivalent to 0.14 of a dog L. D., and if we assume that an equal amount was also retained by the lymph, we have a total of 0.28 dog L. D. Had this quantity not been at the disposal of the animal for further absorption and fixation during the period of incubation the animal would no doubt have survived. But even as matters stand it is interesting to observe that after the injection of so small an overdose of the toxin as that represented by the 1.2 lethal doses actually injected, the very considerable amount of 3,185 guinea-pig units (or 0.5 dog L. D.) had been absorbed in the waiting period of 5 hours and 35 minutes.

Experiment X-16. We may now consider the results obtained in X-16. By this time our experience had shown how important it is to deplete the animal's blood vascular system as sharply as possible at each bleeding. This can be done without danger, by successive withdrawals of approximately half of an animal's blood, because of the ease with which normal dog's blood can be supplied at once by way of the femoral vein. Experiment X-16 is noteworthy for the reason that so many as 16.5 dog lethal doses were injected and that so many as 7.6 dog lethal doses were absorbed and disposed of in an irrecoverable manner in the course of the experiment. The animal's tissues were exposed to the full action of the 16.5 lethal doses of the toxin during the waiting period of 7 hours and 41 minutes. There was further opportunity for an additional but smaller and indeterminate uptake of the toxin during the 2 hours and 35 minutes that were consumed in the ten bleedings and infusions. The "washing-out" in this experiment was so complete that there remained only 442 guinea-pig units in the animal's blood vascular system, out of an injected total of 75,440 units. On the assumption that the lymphatic system also still harbored 442 guinea-pig units, it is evident that we can account, on our balance sheet, for 74,556 guinea-pig units, in the form of recovered and assayable toxin and in the form of fixed toxin.

From an experiment of this kind we cannot, however, conclude that all of the 7.6 lethal doses that had to be charged to fixation were really fixed and not in part destroyed. It was pointed out in Paper V that

the kidneys are not eliminating organs for the toxin to any appreciable extent until a very large excess of it appears in the blood, in which event some of it escapes by the kidneys. Since no data are available in respect to the threshold point at which blood-borne toxin is excreted by the kidneys of dogs, we have assayed the urine of a number of these animals after injecting them intravenously with varying amounts of the toxin. The results are given in Table II. It is evident, in view of the many thousands of guinea-pig doses of toxin that were injected in the experiments there described, that we need not take into account the relatively small loss via the kidneys, when from 10 to 20 lethal doses are used.

It may well be the case that the specifically reacting organs—the central neurons and the neuromuscular substance of the voluntary muscles—of animals that are so relatively insusceptible to the toxin as the dog do not themselves ever fix much more than a full lethal dose, any more than do those of the sheep or the horse; while the other organs of such relatively insusceptible animals, and particularly their viscera, may have the ability, as contrasted with the similar organs of the sheep and the horse, to keep on absorbing and disposing of an excess of the toxin, up to a certain upper limit, as more and more of it is injected. The sensitive guinea-pig, however, an animal that is killed by a minute amount of the toxin, which is disposed of in such a manner that none of it remains in an assayable form in its blood and lymph, is nevertheless capable of disposing of a very large percentage of from 100 to 400 lethal doses, in a manner that defies detection by bio-assay methods. We have made no experiments to determine the point at which the system of the dog ceases to store toxin after the injection of excessive amounts of it. Our various experiments show that 7.6, out of an intravenously injected 16.5 lethal doses, are fixed by the tissues of this animal in the course of eight hours (see Table I). We have no reason to believe, however, that this is anywhere near the upper limit of the storage power of these animals for the toxin. For in one experiment, Z–58, bio-assays of blood collected 24 hours after the injection of 100 dog lethal doses showed that the animal had fixed and otherwise disposed of 58.4 dog lethal doses. The data now at hand are not as yet sufficient to prove or disprove the correctness of the suggestion made above, that the specifically reacting tissues of

TABLE I

Rapidity of fixation of tetanus toxin in the body

DATE	DOG	WEIGHT	DOSE PER KG. IN L.D. 50's	TOTAL DOSE IN		INTERVAL BETWEEN INJECTION OF TOXIN AND FIRST BLEEDING	TOXIN IN BLOOD STREAM AT TIME OF FIRST BLEEDING IN L.D. 50's	NUMBER OF BLEEDINGS AND INFUSIONS OF NORMAL BLOOD	TOXIN REMOVED BY BLEEDING AND INFUSION OF NORMAL BLOOD IN L.D. 50's	TOXIN LEFT IN BLOOD STREAM AFTER LAST BLEEDING		INTERVAL BETWEEN INJECTION OF TOXIN AND LAST BLEEDING	TOXIN BOUND AT TIME OF LAST BLEEDING[a]		PERCENTAGE OF INJECTED TOXIN BOUND AT TIME OF LAST BLEEDING	REMARKS
				L.D. 50's	Dog L.D.'s					L.D. 50's	Dog L.D.'s		L.D. 50's	Dog L.D.'s		
1936		*kg.*													*per cent*	
Apr. 23	X-3	9.09	1,450	13,200	3.02	4 min.	13,000	5	12,608	1,098	0.25	53 min.	(See protocol of this experiment)			April 28, head symptoms of descending tetanus. May 1, difficulty in chewing; tremor of hind legs. May 2, difficulty in chewing and swallowing; salivation; tremor of hind legs more pronounced. May 5, great difficulty in swallowing; no tactile motor tetanus. May 19, stiffness of muscles of head still apparent. Survived
Apr. 23	J-17 (Control for X-3)	10.0	692.5	6,925	1.44											April 25, slight wrinkling of forehead. April 26, well marked head symptoms of descending tetanus. April 27, generalized rigidity. April 28, animal prone; tetanic convulsions. April 29, dead
May 14	X-8	10.9	1,420	15,500	2.96	1 hr. 35 min.	9,080	7	9,068	1,090	0.2	3 hrs. 11 min.	4,250	0.81	27.4	May 19, slight erection of the ears and furrowing of the brow. May 20, wrinkling of brow more marked; eyes drawn; condition otherwise good. May 21, head symptoms of descending tetanus more pronounced. May 24, condition improving. Survived

June 2	X-10	6.9	1,431	9,880	2.98	4 hrs. 41 min.	(See footnote b)	7	6,750	147	0.04	6 hrs. 10 min.	2,840	0.86	28.8	June 7, questionable wrinkling of brow. June 8, definite head symptoms of descending tetanus; severe difficulty in swallowing; slight stiffness of the extremities. June 10, profuse salivation; regurgitates food; stiffness of muscles more pronounced; stumbles when running. June 12, condition improved, although animal is still very stiff and has some difficulty in swallowing. June 15, improving; eats and drinks well; some muscular rigidity still apparent. July 1, well
June 2	X-11 (Control for X-10)	8.0	1,440	11,520	3.0											June 4, wrinkling of brow. June 5, marked head symptoms of descending tetanus; stiffness of gait. June 6, animal prone; tactile motor tetanus. June 8, dead
June 23	X-12	10.0	1,396	13,960	2.9	7 hrs. 40 min.	2,560	7	2,643	159	0.03	9 hrs.	11,000	2.33	80.4	June 26, head symptoms of descending tetanus; ears erect; brow wrinkled; palpebral fissures narrowed; some difficulty in chewing and swallowing; a little stiffness of hind legs. June 28, extreme salivation; generalized muscular rigidity present, but animal can still run about. June 30, prone; convulsive spasms; died at 2:30 P.M.
June 24	X-13 (Control for X-12)	8.18	1,440	11,780	3.0											June 26, definite head symptoms of descending tetanus. June 28, generalized muscular rigidity. June 29, prone. June 30, convulsive spasms; death
1937 May 10	X-14	13.0	583	7,600	1.2	5 hrs. 25 min.	2,889	6	2,707	854	0.14	6 hrs. 27 min.	3,185	0.50	41.6	May 13, slight erection of ears; animal is active and alert. May 14, brow wrinkled. May 15, head symptoms of descending tetanus more pronounced. May 16, beginning stiffness of extremities. May 17, marked head symptoms of descending tetanus; profuse salivation; all extremities moderately stiff; toes spread. May 19, dead

[a] We have assumed that at the time of the last bleeding the lymphatic system contained the same minimal amount of toxin as the blood vascular system.
[b] In Experiment X-10, bio-assays of the first sample of blood were unsatisfactory.

TABLE I—*Concluded*

DATE	DOG	WEIGHT	DOSE PER KG. IN L.D. 50's	TOTAL DOSE IN		INTERVAL BETWEEN INJECTION OF TOXIN AND FIRST BLEEDING	TOXIN IN BLOOD STREAM AT TIME OF FIRST BLEEDING IN L.D. 50's	NUMBER OF BLEEDINGS AND INFUSIONS OF NORMAL BLOOD	TOXIN REMOVED BY BLEEDING AND INFUSION OF NORMAL BLOOD IN L.D. 50's	TOXIN LEFT IN BLOOD STREAM AFTER LAST BLEEDING		INTERVAL BETWEEN INJECTION OF TOXIN AND LAST BLEEDING	TOXIN BOUND AT TIME OF LAST BLEEDING		PERCENTAGE OF INJECTED TOXIN BOUND AT TIME OF LAST BLEEDING	REMARKS
				L.D. 50's	Dog L.D.'s					L.D. 50's	Dog L.D.'s		L.D. 50's	Dog L.D.'s		
1937		*kg.*													*per cent*	
May 12	X-15	13.6	830	11,400	1.7	4 hrs.	7,348	7	6,285	990	0.15	4 hrs. 55 min.	3,135	0.47	27.6	1575 units of tetanus antitoxin were injected intravenously 4 hours and 56 minutes after injection of toxin. No symptoms of tetanus appeared. Animal remained well
Oct. 15	X-16	9.54	7,909	75,440	16.5	7 hrs. 41 min.	21,000	10	39,645	442	0.10	10 hrs. 16 min.	34,951	7.60	46.4	65,450 units of antitoxin were injected intravenously 10 hours and 26 minutes after injection of toxin. Animal remained well and active. No symptoms of tetanus observed
Oct. 15	X-17 (Control for X-16)	8.64	7,900	68,300	16.5											October 16, no evidence of local or general tetanus. October 17, the animal is prone; marked head symptoms of descending tetanus: wrinkling of brow, erection of ears, trismus, opisthotonos; rigidity of all four extremities; frequent tetanic convulsions. October 18, dead
Nov. 4	X-18 (Control for X-16)	11.4	8,050	90,200	16.8											65,450 units of tetanus antitoxin injected intravenously ten hours and 20 minutes after injection of toxin. No symptoms of tetanus developed. Remained well
Dec. 22	X-23	11.4	6,840	78,000	14.3	10 hrs. 53 min.	19,000	11	26,730	400	0.07	14 hrs. 30 min.	50,470	9.25	64.7	This animal died on the operating table while the final samples of blood and lymph were being collected
Dec. 22	X-24 (Control for X-23)	12.7	3,420	43,550	7.1											December 23, malaise, erection of ears, wrinkling of brow. December 24, dead

an animal, that respond to minute amounts of the toxin by giving rise to the two cardinal symptoms of tetanus, may not continue to absorb and fix the toxin at the proportionate rate shown by other organs of the body, such as the viscera, as increasingly large amounts of it are injected.

It is also impossible to give a definite answer to the question whether these non-specifically affected organs that actually store so large an excess of the toxin, over what is needed to kill an animal, are or are not endowed with the property of destroying this excess. But were this found to be the case, it could not be concluded that this destructive process goes on without damaging the organs in which it takes place. The interesting fact emerges from our experiments[10] that in both the sensitive and the insensitive animals only a relatively small fraction of the lowest lethal dose of the toxin is fixed by the specifically reacting organs, in comparison with the large amount that can be stored by the non-specifically reacting organs, and that the relatively small amount fixed by the former organs, out of the lowest injected lethal dose, always leads to death.

Our series of experiments show that the tissues of the relatively insusceptible dog begin to fix the toxin from the very moment at which it escapes from the capillaries and comes into contact with their cells. Even the tissues of Dog X-3 had certainly fixed a small fraction of 3 lethal doses of blood-borne toxin in the short interval of four minutes during which they were exposed to its full action. And again in experiment X-8, in which the animal was also exposed to the full action of 3 lethal doses, the dog's tissues had fixed in one hour and 45 minutes 0.81 of a dog lethal dose. In the short interval of time involved in this experiment the toxin was so firmly fixed that seven bleedings, in each of which 35 per cent of the animal's blood was withdrawn, were insufficient to dislodge it.

Experiments of this kind appear to show that our toxin is irreversibly fixed by the specifically reacting organs, at least, from the moment it reaches their cells, and that there is no preliminary period of a loose or reversible absorption. We may make the permissible assumption that its fixation there is directly proportional to the concentration gradient between the outside and inside of the cells. This assumption

[10] See Papers III, V, VI.

TABLE II

Renal excretion of intravenously injected tetanus toxin

DOG	WEIGHT	DOSE		INTERVAL BETWEEN INJECTION OF TOXIN AND COLLECTION OF URINE	GUINEA-PIG	AMOUNT OF URINE INJECTED INTRAMUSCULARLY INTO GUINEA-PIGS	FATE OF INJECTED GUINEA-PIGS	FATE OF DOGS AND REMARKS
		G.P. L.D. 50's	Dog L.D.'s					
	kg.					*cc.*		
Z-50	11.2	53,500	10.0	2 hours 5 minutes	a	1.0	No symptoms of tetanus	Dog Z-50 showed the first symptoms of tetanus 36 hours after injection of toxin: malaise, erection of ears, furrowing of brow. A large dose of antitetanic serum (70,000 units) was injected intravenously as soon as these manifestations were observed. The dog succumbed to the disease on the third day after injection of toxin
					b	2.0	No symptoms of tetanus	
					c	3.0	No symptoms of tetanus	
				7 hours 55 minutes	d	1.0	Mild local tetanus. Survived	
					e	2.0	Mild local tetanus. Survived	
					f	4.0	Died of tetanus on ninth day	
Z-52	7.04	67,500	20.0	6 hours 30 minutes	g	5.0	Succumbed to tetanus in five days	Dog Z-52 was killed by another dog on the second day after injection of toxin
Z-59	8.6	82,450	20.0	2 hours 40 minutes	l	3.0	Mild local tetanus. Survived	Dog Z-59 had severe descending tetanus 48 hours after the injection of toxin. Killed by intravenous injection of air
					m	3.0	Mild local tetanus. Survived	
				8 hours 25 minutes	n	3.0	Died of tetanus in four days	
				24 hours	o	1.2	Mild local tetanus. Survived	
Z-60*	10.7	102,100	20.0	2 hours	p	1.0	Mild local tetanus. Survived	Dog Z-60 died of tetanus 22 hours after the injection of the toxin
					q	2.0	Mild local tetanus. Survived	
					r	4.0	Advanced local tetanus. Survived	
				8 hours 30 minutes	s	1.0	Mild local tetanus. Survived	
					t	2.0	Mild local tetanus. Survived	
					u	4.0	Mild local tetanus. Survived	
				24 hours	v	1.0	Mild local tetanus. Survived	
					w	2.0	Mild local tetanus. Survived	
					x	4.0	Mild local tetanus. Survived	

Z-56	9.2	442,500	100.0	4 hours 25 minutes	h	2.0	Died of tetanus in six days	Dog Z-56 died 48 hours after injection of toxin
					i	4.0	Died of tetanus in three days	
Z-58	6.4	307,450	100.0	3 hours 40 minutes	j	5.0	Died of tetanus in two days	Dog Z-58 showed the first symptoms of tetanus 22 hours after the injection of toxin: malaise, erection of ears, awkward gait. Died in 46 hours
				24 hours	k	4.4	Died of tetanus in four days	

It is evident that the toxin appears in the urine of dogs, in assayable amounts, seven and eight hours after the intravenous injection into these relatively insusceptible animals of 10 to 20 lethal doses (53,500 and 67,500 guinea-pig units). Even the injection of so large an amount as 100 dog lethal doses (442,500 guinea-pig units) was followed by the excretion of a negligible quantity of the toxin; 2 cc. of urine collected four and a half hours later contained less than one guinea-pig unit. The above assays of the toxin content of the urine corroborate the findings of earlier writers that the threshold for the urinary secretion of the toxin is surmounted only when excessively large quantities are present in the blood. The urine of two horses that received a fatal dose of the toxin was tested daily on guinea-pigs, in 3 to 4 cc. portions, with entirely negative results (Table III, Paper V).

* In this experiment, immediately before the injection of the toxin, the bladder was emptied by means of a catheter, with the expected result that the first sample of urine, collected two hours later, contained slightly more toxin per cubic centimeter than the subsequent samples.

may be taken to hold also for the many other organ systems of the body, but their relative threshold for the uptake of the toxin, and their singular capacity to dispose of a very large number of lethal doses, must not be left out of consideration.

Had we injected intravenously 16.5 lethal doses of the toxin into a dog, as was done in experiment X-16, and had we then subjected it (after an interval of 15 to 20 minutes between the injection of the toxin and the first withdrawal of blood) to ten successive sharp bleedings and infusions, we consider it not unlikely that the animal's central neurons and the end-plates of its voluntary muscles would have absorbed and fixed, in this short interval of time, that minute fraction of one lethal dose of toxin that inevitably leads to death. In any event, the rapidity with which a lethal dose of the toxin is fixed in the body after injection of amounts varying from 1.2 to 16.5 lethal doses, certainly proves that the toxin is carried to the neurons of the central nervous system, not to speak of other parts of the body, *by the blood vascular system.* This rapid uptake and binding of blood-borne toxin by the motor cells of the spinal cord, medulla and pons cerebri, to speak here of these only, is irreconcilable with the theory that this poison, in contrast to all others, is carried to them, in some mysterious manner, and in an indeterminate time, by the axis cylinders of motor nerves; and that an intervening, so-called brain-blood barrier prevents the blood stream from bringing it to them. The fact that the much larger molecules of an antitetanic serum can reach and neutralize the toxin that has been fixed by these motor nuclei, by way of the blood stream, when the serum is used at any time during the incubation period proper, also proves that these long-held beliefs are no longer tenable.

FURTHER EXPERIMENTS ON THE PARTITION OF TOXIN BETWEEN THE BLOOD AND LYMPH

The equipartition problem has occupied our attention for a number of years. It was fully discussed in Paper V, in which will be found reports of many experiments proving that, in sheep, the toxin in the lymph must have been practically in equilibrium with that of the blood from twenty-four hours on, if not earlier. It was not feasible for various reasons to assay the lymph of sheep for its toxin content

earlier than forty-eight hours after intravenous injection, whereas arrangements should have been made, as we can now see, for making the lymph assays both before and after the seven-hour "waiting period." A careful study, however, of the many blood-toxin assays of Sheep No. 4, as given in Table IV of Paper V, shows, in the light of our later experiments with dogs, that equilibrium was probably established, in that animal, as early as seven hours after the injection of the toxin. At this time 1.5 cc. of the blood of this sheep contained 1 guinea-pig median lethal dose of the toxin. When this 7-hour blood-toxin assay is properly appraised in the light of the similar assays found for the subsequent 24, 48, 72, 96, 120 and 144-hour periods, one cannot doubt that the toxin content of the lymph of this sheep was at least very approximately equal to that of the blood as early as the seventh hour. The extent to which the sheep and the horse can fix and store the toxin is strikingly different from that shown by the dog and certain other animals, as was pointed out in Paper V. When, for example, from 2 to 10 lethal doses are injected into sheep (and we have not experimented with larger doses) it is found that only one lethal dose is fixed and stored by their tissues, the excess being equally divided, or approximately so, between the cardiovascular and lymphatic systems. Further observations reported in this paper show that the injection of an increasingly larger amount of toxin (1 to 100 lethal doses) into dogs is followed by an increasingly larger fixation and disposal of it. As soon, however, as the maximum degree of fixation has been attained in a given case, the excess of non-fixed and recoverable toxin is found to be nearly equally divided between the blood and the lymph, exactly as in the case of the sheep, whose comparative ability to store the toxin is so much smaller. We have not yet determined the upper limits of this capacity of the dog's tissues to dispose, in a non-recoverable manner, of larger amounts than 100 lethal doses of the toxin.

The passage of the toxin from the blood to the lymph, and the fact that the equipartition between the two fluids is soon effected in the dog, is clearly shown by the following experiment (X-19). Tetanus toxin amounting to 14.3 dog lethal doses (77,000 guinea-pig L.D. 50's) was injected intravenously into a dog (X-19) weighing 11.4 kg. Eight hours later the thoracic duct was cannulated under ether

anesthesia and several cubic centimeters of lymph were collected in a flask containing a few drops of 2.5 per cent sodium citrate solution, as anticoagulant. A sample of blood was then removed from the external jugular vein and also citrated. The toxiferous blood and lymph were diluted with sterile broth, in the ratio of 1 to 19, and were then injected in graduated doses into guinea-pigs. The results of the assays showed that 0.049 cc. of citrated blood contained one guinea-pig unit of the toxin, while in the citrated lymph this amount of toxin was present in 0.048 cc. If the blood volume of the dog be taken as 1,140 cc. and the lymph volume be assumed to equal that of the blood, the total number of guinea-pig median lethal doses of toxin present in both the blood vascular and lymphatic systems of dog X-19, eight hours after the intravenous injection of 77,000 guinea-pig units, is equal to 1140/0.049 (blood) + 1140/0.048 (lymph) = 47,010 guinea-pig units, or 8.6 dog lethal doses. In other words, 60 per cent of the injected 14.3 lethal doses of toxin remained unbound after eight hours; one-half of this 60 per cent was present in the blood, the other half in the lymph.

The amount of toxin fixed in eight hours by the tissues of the animal was therefore 14.3 − 8.6 = 5.7 dog lethal doses, or 40 per cent of the injected dose of 14.3 dog lethal doses. Not only, therefore, had the lymph come into equilibrium with the blood, in respect to its toxin content, in the course of the eight hours, but the body had also fixed a maximum percentage of the toxin in that time interval. The results of this experiment (X-19) are in excellent agreement with those obtained in X-16, as given in the detailed protocols of that experiment. It can be calculated from the data there given that equipartition of the toxin between the blood and lymph, after the injection of 16.5 dog lethal doses, was established in 7 hours and 41 minutes, and that 43 per cent of the toxin had been fixed in the body of the animal in that time. In the present experiment, with dog X-19, in which the somewhat smaller dose of 14.3 dog lethal doses was employed, equipartition of the toxin between the blood and lymph was completed in eight hours, and 40 per cent of the injected toxin was fixed in the body in this time.

The results of experiment X-19, as well as those of the further experiments, X-20 and X-22, on the partition of toxin between the blood and lymph are summarized in Table III. In experiment X-20,

TABLE III

The partition of toxin between the blood and the lymph following the intravenous injection of large doses

DOG	WEIGHT	DOSE		INTERVAL BETWEEN INJECTION OF TOXIN AND COLLECTION OF LYMPH	AMOUNT OF LYMPH CONTAINING 1 G.P. L.D. 50	AMOUNT OF TOXIN IN LYMPHATIC SYSTEM[a]		INTERVAL BETWEEN INJECTION OF TOXIN AND COLLECTION OF BLOOD	AMOUNT OF BLOOD CONTAINING 1 G.P. L.D. 50	AMOUNT OF TOXIN IN BLOOD VASCULAR SYSTEM		AMOUNT OF TOXIN BOUND DOG L.D.'s	REMARKS
		G.P. L.D. 50's	DOG L.D.'s			G.P. L.D. 50's	Dog L.D.'s			G.P. L.D. 50's	Dog L.D.'s		
	kg.				*cc.*				*cc.*				
X-19	11.4	77,000	14.1	8 hrs. 30 min. (Thoracic duct lymph. Animal in resting condition)	0.048	23,750	4.35	8 hrs. 10 min.	0.049	23,360	4.26	5.5	Died 50 hours after injection of toxin. No gross pathological changes found at autopsy
X-20	15.5	79,100	10.7	8 hrs. 58 min. (Thoracic duct lymph. Animal in resting condition)	0.039	39,600	5.36	8 hrs. 10 min.	0.047	32,980	4.46	0.9[b]	50 hours after injection of toxin definite symptoms observed: erection of ears, furrowing of brow, slight stiffness of hind legs. Died on the 4th day
								29 hrs. 15 min.	0.125	12,400	7.3	7.3	
				9 hrs 13 min. (Lymph from left cervical lymphatic trunk)	0.1			56 hrs.	0.38	4,078	0.4	9.5	
X-22	10.9	86,250	16.5	7 hrs. 50 min. (Thoracic duct lymph. Animal in resting condition)	0.063	17,300	3.31		(See footnote c)			9.88[d]	Succumbed to tetanus 50 hours after injection of toxin
				8 hrs. (Thoracic duct lymph. Hind legs exercised)	0.065	16,800	3.21					10.08[d]	
				8 hrs. 15 min. (Lymph from left cervical lymphatic trunk)	0.12								

[a] It is assumed that the total volume of lymph equals that of the blood.

[b] We cannot explain why this animal differed so strikingly from the others of our series (Cf. Table I of Paper VII) in fixing only 0.9 dog L.D.'s in the 9 hours that followed the injection of 10.7 dog L.D.'s. Later, however, like all of the other dogs studied, it disposed of the greater part of the injected dose.

[c] Assays of the blood for toxin could not be carried out because a sufficient number of guinea-pigs was not available.

[d] Equipartition of toxin between the blood and the lymph is assumed.

a dog weighing 15.5 kg. was injected intravenously with 79,100 guinea-pig units (10.7 dog lethal doses). The assays of the blood and thoracic duct lymph collected eight hours and ten minutes later revealed that the two fluids were approximately in equilibrium in respect to their toxin content; 0.047 cc. of blood contained one guinea-pig unit of toxin, while the same amount of toxin was contained in 0.039 cc. of lymph. The toxin content of the lymph obtained from the left cervical lymphatic trunk, however, was less than half that of the thoracic duct lymph. Nevertheless, we cannot be far wrong if we assume that the average lymph was approximately as toxic as the blood. Incidentally, as shown by the bio-assays of blood collected 56 hours after the injection of toxin, dog X-20 fixed, in an irrecoverable manner, and otherwise disposed of 9.5 of the injected total of 10.7 dog lethal doses. Thus, it would appear that the dog, like other relatively insensitive animals, such as the rabbit, has the ability to fix and destroy many lethal doses of toxin.

Experiment X-22 was performed with the purpose of determining the effect of muscular exercise upon the toxin content of the thoracic duct lymph and of confirming the interesting observation made in X-20, that lymph collected from the left cervical lymphatic trunk, which drains the left half of the head and neck and the left foreleg, harbors only half as much toxin as the thoracic duct lymph. In this experiment, in which the dog had not been deprived of food, the lymph had a milky appearance and flowed from the thoracic duct at the rate of 6.5 cc. in five minutes, with the animal tied to the operating table and under ether anesthesia. This rate of flow is materially higher than that observed in fasting dogs, in which the rate is 3 to 4 cc. in five minutes. After collecting the "resting" lymph, we exercised the hind legs of the animal under discussion, in the hope that we could thereby obtain a mixed sample made up of "visceral" lymph and also of lymph originating in the passively exercised extremities. That there is no flow of lymph from the extremities of a resting dog has been clearly demonstrated by the admirable experiments of Drinker and Field. A few seconds after the passive movements were initiated, a sharp increase was noted in the flow of lymph from the thoracic duct. The amount collected in five minutes was 11.0 cc., which represents an increase of 70 per cent over the amount obtained in the

absence of passive movements. Part of this increase was doubtless due to the addition to the visceral lymph of leg lymph driven into the thoracic duct by the massaging action of the exercised muscles. Lesser, many years ago, made the interesting observation that passive exercise of the hind legs of a dog not only resulted in an augmentation of the flow of thoracic duct lymph but also influenced its physical characteristics. While his dog was being passively exercised, the lymph partly lost its milky appearance and became more limpid. This change he attributed to the admixture of clear lymph from the extremities to the chyle. Our finding that "exercise" lymph is slightly less toxic than "resting" lymph supports this explanation, for it appears that peripheral lymph contains less toxin per cubic centimeter than the lymph originating in the thoracic and abdominal viscera. Thus, as indicated in Table III, the cervical trunk lymph of dogs X-20 and X-22 contained only about half as much toxin per cubic centimeter as the thoracic duct lymph. It is obvious that the addition of any considerable amount of this clear, relatively toxin-poor lymph to the more highly toxic visceral lymph would render it less toxic; the results of experiment X-22, in which the bio-assays revealed a definite, though slight, loss of toxicity of lymph collected following exercise, fulfill our expectations. These studies lead us to the conclusion that several hours after the intravenous injection of toxin the thoracic duct lymph is slightly more toxic than the blood and that the peripheral lymph is only half as toxic, and so, we can safely assume, for the present, that an approximate equipartition of toxin between the blood and lymph, in the sense that equal volumes contain the same amount of toxin, obtains under the conditions stated.

2. The Return Passage of Toxin from the Lymph to the Blood

We may now consider the reverse passage of the toxin from the lymph to the blood, under the experimental conditions that obtained in dog X-16. An examination of the data given in the protocol of that experiment shows that there were 21,000 guinea-pig units of toxin in the blood of the animal at the time of the first bleeding (7 hours and 41 minutes after the injection of the toxin) and it may safely be concluded, in view of the experimental data given above and in Paper V, that at this time the lymphatic system harbored an equal

amount of the toxin. Now, our assays of the removed blood show that a total amount of 39,645 guinea-pig units of toxin was taken out of the animal in the course of the ten bleedings and reinfusions. This large total of toxin that was actually removed from the animal's *blood vascular system* is nearly double the 21,000 units shown to have been present there at the start, before the first of the ten bleedings took place. The recovered excess of toxin is 18,645 guinea-pig units (39,645 minus 21,000). What is the source of this newly arising surplus of toxin?

We have demonstrated that the toxin which has been fixed upon the tissues cannot be removed by repeatedly perfusing them with normal blood, and also that the inoculation of test animals with emulsions of such tissues (free from included toxiferous blood and lymph) is powerless to induce even the slightest symptoms of tetanus. It cannot therefore be thought that the newly arising surplus of toxin merely represents a large part of the 34,951 guinea-pig units fixed in the body prior to the first bleeding, that had again been liberated from the tissues in the course of the ten "washings" with normal blood. The protocols of our experiments show very clearly that a supposition of this kind is untenable for the sound reason that we have never been able to save a dog that had fixed as little as one lethal dose out of an injected total of two or three, when as many as seven bleedings and infusions were carried out.

It is evident, then, that the excess of 18,645 guinea-pig units found in the total recovered amount of 39,645 units, of which only 21,000 were present in the blood at the time of the first bleeding (when equipartition had been established), must have come from that other great reservoir in the body of freely moving and unbound toxin—the lymphatic system.

Experiment X-23. Another "wash-out" experiment (X-23) was carried out with the object of studying further the conditions that influence the reverse passage of toxin from the lymphatic to the hemal capillaries. Assays were made of the lymph collected at the time of the first and the last bleedings, in addition to the usual assays of the toxin content of samples of blood. Unfortunately, the subject of this experiment had been starved by mistake for a period of 48 hours, instead of 24, prior to the first bleeding and so tolerated the operative

procedures badly, dying a few minutes after the last bleeding. Furthermore, the experiment was unduly prolonged because of troublesome clotting of the infused blood, which had not been adequately heparinized. Nevertheless, we succeeded in completing eleven bleedings and infusions and in obtaining satisfactory samples of lymph and blood.

The dog (weight 11.4 kg.) was injected intravenously with 78,000 guinea-pig units (14.3 dog L.D.'s) of toxin and was left undisturbed until 10 hours later, when it was anesthetized with ether and subjected to the following operative procedures: The thoracic duct and the left cervical lymphatic trunk were exposed. Then the left femoral artery and vein were cannulated. Distention of the thoracic duct and the cervical trunk was effected by the simple device of compressing them temporarily by raising loops of silk thread passed beneath them; samples of lymph were then collected from each of the vessels by means of a hypodermic syringe. Neither vessel was appreciably injured by this procedure, the tiny puncture wounds closing promptly after withdrawal of the needle. Immediately thereafter the arterial cannula was unclamped until 500 cc. of blood were collected. An approximately equal volume of normal blood was then promptly infused into the femoral vein. The operations of bleeding and transfusing with normal blood were repeated eleven times in all. Respirations ceased while the final samples of blood and lymph were being collected. As indicated above, the unintentional preoperative starvation and the numerous delays that occurred during the bleedings and infusions as the result of the clotting of the infused blood were doubtless responsible for the untimely death of the dog.

The bio-assays of the toxin content of samples of blood reveal that at the time of the first bleeding, 10 hours and 53 minutes after the intravenous injection of so large an amount as 78,000 guinea-pig units, the blood vascular system contained only 19,000 units of the toxin. In the course of the eleven bleedings and infusions a total of 26,730 units was recovered and only 400 guinea-pig units (0.07 dog L.D.) were left in the circulating blood, if its volume equals 10 per cent of the weight of the dog. Assuming that the lymphatic system also harbored 400 guinea-pig units at the end of the experiment, we obtain,

by subtracting the total of the toxin removed in the eleven bleedings (26,730 units), plus the 800 units left in the circulating blood and lymph, from the number of guinea-pig units (78,000) originally injected, 50,470 as the number of units bound by the tissues of the dog (9.25 dog L.D.'s). Thus, in approximately eleven hours the tissues of the animal disposed of 64.7 per cent of the injected 14.3 dog lethal doses (in comparison with dog X-16, in which 7.6 out of the injected 16.5 dog lethal doses were bound in eight hours). In the present experiment 7,730 guinea-pig units of toxin passed from the lymphatic capillaries to the hemal capillaries in the course of the bleedings and infusions, while in experiment X-16, 18,645 units of toxin took this reverse course.

Assays of the lymph removed from dog X-23, which had been deprived of food for two days, showed that a marked decrease in the toxicity of the lymph had occurred in the course of the bleedings and infusions. The thoracic duct lymph contained at the time of the first bleeding approximately two-thirds as much toxin per cubic centimeter as the first sample of blood, and the cervical trunk lymph only one-third as much. The bio-assays of the final samples of blood and lymph revealed that the toxicity of the former was reduced to 2 per cent of its original value, while that of the latter was diminished to 10 per cent. We believe, however, that this lymph did not accurately represent the composition of the lymph in the peripheral capillary net-works, where the reverse passage of toxin took place, for it was really a sample of the relatively stagnant and hence more highly toxiferous lymph that had been retained in the receptaculum chyli and the thoracic duct. The unlooked-for death of the animal prevented us from obtaining a larger and more truly representative terminal sample of lymph. There can be little doubt that the toxicity of such a sample would have come very close to equalling that of the final blood. Despite its faults and shortcomings, however, this experiment bears out our explanation of the recovery by the "wash-out" method of a large surplus of toxin over the amount originally present in the circulating blood: that the toxin diffused through the exceedingly thin walls of the lymphatic capillaries, traversed the minute "tissue spaces," and entered the hemal capillaries, whence it was removed in the bleedings.

A study of the protocols of the experiments made with dog X-16 shows how rapidly the toxin returned from the lymphatic to the blood vascular system as the toxin "pressure" in the latter system was steadily being lowered by the repeated bleedings. At the first bleeding, nearly half the number of guinea-pig units of toxin known to be present in the blood was removed, and in the subsequent bleedings the process of depleting the body of both blood- and lymph-borne toxin continued. It is not surprising, then, that the lymph-borne toxin, originally in equilibrium, in our sense of the term, with that present in the blood vascular system prior to the bleedings, should pass through the very thin-walled and innumerable lymphatic capillary net-works of the body, to enter by way of the excessively minute "tissue spaces" the greatly toxin-depleted blood capillaries, within which the toxin "pressure" was being steadily reduced to a minimum level. This reverse passage of the toxin through the innumerable lymphatic capillary net-works of the body continued until the bleeding and reinfusing experiments were concluded, at which time 88 per cent of the 21,000 guinea-pig units of toxin, calculated to have been present in the lymphatic system at the time of the first bleeding, had been transferred to the blood stream. We have every reason to believe that if more bleedings and reinfusions had been carried out still more of the lymph-borne toxin would have passed into the blood, so that a complete equipartition would finally have been established, in case this had not already been completed in the 2 hours and 15 minutes consumed in the experiment. But even as matters stand we can account for the reverse passage of 18,480 of the 21,000 units calculated to have been present in the lymphatic system. If we add to this number of units of toxin the amount that was retained by the lymphatics at the close of the ten bleedings, we can account for 18,645 units out of the 21,000 originally present.

In stating that the lymph-borne toxin returned to the blood vascular system by way of the capillary lymphatic net-works we have not considered the extent to which lymph was carried to the venous blood via the main lymphatic trunks. Only a relatively small amount of the toxiferous lymph could have been returned to the blood stream in this way in the course of our experiments with anesthetized and resting animals. For example, the amount of lymph that flowed from

the cannulated thoracic duct of dog X-19, in five minutes before the first bleeding was undertaken, amounted to 3 cc., and this rate of flow undoubtedly decreased as the experiment went on. It is evident also from the data given in respect to the lymph flow from the thoracic duct and left cervical trunk of dog X-16 that only a small quantity of toxiferous lymph, amounting probably to not more than 100 cc., could have been transferred to the blood in the course of the experiment by way of the two main lymphatic ducts. The reverse passage of the toxin from the lymphatic to the blood vascular system must therefore have been effected almost entirely by way of lymphatic net-works, which are so numerous in all regions where blood capillaries are found; and this return of the toxin took place most largely, no doubt, by way of the lymphatic net-works of the viscera.

3. The Return Passage of Toxin via the Lymphatic Capillaries Gives Us a Method of Determining, Approximately, the Lymph Volume in the Closed Lymphatic System of Resting Dogs

That the reverse passage of toxin from the lymphatic to the blood vascular system may serve as the basis of a method of approximately determining the lymph volume of a resting animal is shown by the results of all of our "wash-out" experiments, and particularly by those of experiments X-16 and X-23. In both of these experiments, as far as could be determined by our bio-assays, all but a negligible fraction of the total amount of toxin that was harbored by the entire lymphatic system was recovered. Our ideas in respect to the total volume of lymph, *in the closed lymphatic system*, appear to rest only upon conjecture. The literature of the subject gives no evidence that these conjectures are based on valid experiments of one kind or another. Delamare states that Krause believed that the volume of lymph amounts to $\frac{1}{3}$ of the body weight, that Ludwig and Magendie put it at $\frac{1}{4}$, and that Bidder recorded it as amounting, in the case of the cat, to 1/5.36, and in the case of the dog to 1/6.66 of the body weight. From a horse Schmidt obtained in 24 hours an amount of lymph equal to the total amount of its blood. Other authors also have collected very large quantities of lymph from other animals, as well as from man. It is evident that from data of this kind no definite conclusion can be drawn in respect to the total volume of lymph as compared to that of the blood.

Many considerations indicate that the total volume of lymph present in the entire lymphatic system, inclusive of the lymphatic glands, Peyer's patches, and other adnexa, is subject, temporarily, to wider fluctuations than is the case with the total volume of blood. The latter would appear on physiological grounds to be more conservatively maintained. In attempting to estimate the total volume of the lymph of a resting and anesthetized animal by our method we have been hampered by our inability to determine the true median toxin content of the mixed lymph of the body. Nevertheless, our method enables us to determine both the upper and the lower limits of the calculated values of lymph contained in the closed lymphatic system. If the higher toxin content per cubic centimeter of thoracic duct lymph is taken as the standard value for the toxin content of the mixed lymph, our calculation gives us a minimum figure for the total lymph volume. If, on the other hand, we take the toxin content per cubic centimeter of the lymph flowing from the left cervical lymphatic duct, which gives us a sample of the peripheral as distinguished from the visceral lymph, our calculation gives us a maximum figure for the lymph volume.

Since in a resting, anesthetized and fed animal, or, in a similar animal that has not been fed, the greater part of the lymph present in the closed lymphatic system is contained in the viscera, it is evident that any figures for the total lymph volume that are based on the toxin content per cubic centimeter, of the visceral lymph, will give a truer estimate of the total lymph volume than can be derived from figures based on the toxin content of the smaller volume of lymph present in the quiescent muscles of the body. And, since the visceral lymph contains twice as much toxin per cubic centimeter as the peripheral lymph (cervical trunk lymph), the figures for the total volume of lymph, arrived at by the two calculations, will be as 1 is to 2; the true values will lie somewhere between, but will be nearer the first than the second.

The results of the "wash-out" experiments made with dog X-16 indicate that the lymph volume of the animal was approximately 0.9 that of its blood volume, when the calculation is made on the basis of the toxin content of the thoracic duct lymph; the lymph volume calculated on the basis of the toxin content of the cervical lymph

comes to 1.8 times the blood volume. The varying conditions that obtain in experiments of this kind must be borne in mind. The results obtained in a similar experiment, with dog X-23, that had been starved for 48 hours prior to the injection of the toxin, indicate that its lymph volume was materially lower than that of the former animal. The minimum and maximum values for the total lymph volume of this dog, derived in the manner described above, are 0.7 and 1.4 times the blood volume. The experimental data on which the above calculations are based will be found in Tables I and III.

Further trials of our method must be made in order to determine whether such variations in the lymph volume of resting dogs actually occur when the animals are subjected to the different conditions described in our protocols. We hope that when tetanus toxin is replaced by diphtheria antitoxin in our "wash-out" experiments we shall have at our disposal a more accurate and dependable method for determining the lymph volume in the closed lymphatic system of animals, both in the resting and the active condition. Erin Madsen has shown that the use of diphtheria antitoxin furnishes a method of determining very accurately the blood volume of man. The sensitive intracutaneous test permits of the detection of so small a quantity of this antibody as 0.00005 of a unit. The accuracy and ease with which this antitoxin can be quantitatively estimated would make it especially suitable for our purpose, since the blood volume of our animals could be determined at the same time and the quantitative partition of this antitoxin between the blood and lymph could also be determined with a much greater accuracy than by the use of tetanus toxin or antitoxin. We hope to make use of the reverse passage of diphtheria antitoxin from the lymphatic to the blood vascular system for determining the lymph volume of animals under varying conditions. But before we can get around to this problem we must first conclude our researches on several problems of tetanus on which we are now engaged.

We hold that the toxin escapes from and also enters both blood and lymph capillaries by the process of diffusion. It must be borne in mind that, even when many thousands of guinea-pig units are injected into an animal, we are dealing with only a small amount of this very potent substance. That the toxin is one of the most deadly of all

known poisons is proved by the fact that a very minute amount of an impure but highly toxic culture broth from the *clostridium tetani* suffices to kill test animals such as mice and guinea-pigs. The reverse passage by diffusion from the lymphatic to the blood capillaries takes place in the innumerable lymphatic net-works of the body, and probably to the greatest extent in those of the viscera, as we have already suggested. On the other hand, the anatomical structure of the lymph glands and nodes is such that any toxin there present can diffuse into their blood capillaries in the course of the "wash-out" experiments, at the time the toxin content of the blood vascular system is being steadily lowered.

The total surface area of the lymphatic capillaries and of the smaller lymphatic vessels that have no valves must be very great. When fully distended, their diameter is many times that of the blood capillaries, from which it may be inferred that, under circumstances that often arise, this distention enables the lymphatic system to retain in its net-works, for a time at least, a much larger volume of lymph than is usually supposed to be the case.

That the "interstitial fluid," for example, of the largest peripheral nerve trunks of the voluntary muscles and of the spinal cord, of toxin-injected animals, does not contain toxin in the free and assayable state, when several doses of it have been injected intravenously, has been abundantly proved.[11] That this indetectable toxin was, however, actually fixed upon the voluntary muscles and the gray matter of the spinal cord is clearly proved by the appearance, after the usual period of incubation, of both the peripherally induced muscular and the centrally induced reflex symptoms of descending tetanus. We therefore conclude that, while it may be granted that excessively thin layers of interstitial fluid may be allocated to the normal tissues, the chemical properties of our toxin, which does not induce edema, determine that it shall be fixed upon the adjoining organized structures, whether these be of cellular or other character, rather than that it shall be retained by the postulated molecular layers of fluid in contact with these structures. Under abnormal conditions, however, as in the edematous state induced by the administration of an excess of sodium

[11] See Paper V, pages 366 and 367.

chloride, in which there is actual proof of the presence of a newly produced excess of mobile interstitial fluid (for which room is found in the connective tissues), we may expect to find some free toxin in the edema fluid as long as it continues to be present in the blood and lymph.

The admirable experiments of McMaster and Parsons on the path of escape of vital dyes from the lymphatics into the tissues, as recently described and interpreted by these authors, show very clearly that, before the onset of the mild edema which is eventually induced, the escaping dye "appears to be fixed either between or upon preformed tissue elements." After the onset of the edema, however, the dye that traverses the walls of the lymphatic capillaries is dispersed in the edema fluid. We would cite the following statement with which McMaster and Parsons conclude the account of their numerous and consistent experiments: "The findings strongly suggest that the dyes first extend along the interface present between the surfaces of contiguous connective tissue fibers or along that between connective tissue fibers and an interstitial gel. However, this may be, it is certain that the dyes move through the tissues along tracks, as it were, determined by the form and situation of the connective tissue fibers."

Were our toxin endowed with the property of inducing edema, as is the case with the pontamine sky blue and patent blue V employed by these investigators, we should expect to find that, when given in supralethal amounts, it is not only fixed in the usual manner on the specifically reacting cells of the body, such as those of the anterior horns of the spinal cord and the end-plates of the voluntary muscles, but that a part of the non-fixed excess would be held in solution by the newly formed interstitial edema fluid, as was the case in the experiments with the vital dyes just referred to.

The well known, ingenious experiments of E. R. and E. L. Clark, in which the relation of the living lymphatic vessels of the rabbit's ear to the tissue spaces was studied, have led these authors to express grave doubts concerning the validity of the prevailing belief that free fluid is normally present in the tissue spaces, as is shown in the following statements cited from one of their papers: " . . . under normal circumstances all our evidence appears to indicate that there is no free

fluid present in the tissue spaces," and further, "The results of the direct microscopic observation of the lymphatic capillaries and of the connective tissue, as viewed through a transparent double-walled window introduced in the living mammal, show the presence normally of intact membranes lining both blood and lymphatic vessels and separating their fluid contents from the tissue outside, and the absence, under normal conditions, of free fluid in the tissue spaces."

We cannot undertake to discuss in this paper the very extensive literature pertaining to the whole subject of extracellular fluid—a literature that includes many valuable studies by clinicians as well as by experimental scientists.

Summary

When an animal has been injected with one or more lethal doses of tetanus toxin, the time of its death is determined by that fraction of the toxin that has been fixed by the specifically reacting organs of the body in the first few hours during which they were exposed to the requisite threshold concentration of the poison. Neither the bio-assay nor any other method now at our disposal enables us to detect and assay this fixed fraction of the injected toxin. The results of tests made with the highly sensitive guinea-pig or mouse give us definite information in respect to the amount of the non-fixed toxin that continues to circulate in the blood and lymph. The use of bio-assays in conjunction with other experimental procedures described in the present paper also enables us to determine how soon after the intravenous injection the toxin begins to be fixed by the specifically reacting as well as by the non-specifically reacting organs of the body, and how long a time elapses before the fixation has reached its maximum.

1. Our protocols show that, when three lethal doses of the toxin were injected intravenously, the irreversible fixation of a lethal dose was completed in the relatively short time of 5 to 7 hours. This and the other facts enumerated below were established with the help of the "wash-out" method, details of which are to be found in the protocols of our experiments. As the result of an extensive use of this method we had animals in hand that had fixed a lethal or more than a lethal

fraction of the injected toxin, while all of the non-fixed, still assayable and free toxin present in their blood and lymph, had been removed with the exception of an insignificant and immaterial residue.

2. The results of our experiments show that the toxin begins to be fixed in an irreversible manner from the moment it reaches, via the blood stream, the motor neurons of the central nervous system and the end-plates of the voluntary muscles. When not enough time was allowed to elapse for the fixation of a full lethal dose of the toxin by these specifically reacting structures, the injected and washed-out animals responded with a milder form of descending tetanus, from which they recovered. Experiments of this character prove that there is no brain-blood barrier that stands in the way of the steadily progressing uptake and fixation of blood-borne toxin by central motor neurons. The toxin is therefore brought to the motor neurons of the spinal cord, medulla and pons and to all other parts of the body, in all cases of general as distinguished from a purely local tetanus (which is caused by lymph-borne toxin), solely by way of the blood stream. This experimentally determined fact cannot be reconciled with the prevailing belief that the toxin is carried in some mysterious manner, and in an indeterminate time, to the central neurons by way of the axis cylinders of motor nerves.

3. The striking observation was made that when a dog is given time to fix a lethal fraction of several intravenously injected dog lethal doses, and is then deprived of the free circulating toxin by the method of viviperfusion with whole blood, the injection of a relatively large dose of antitoxin during the period of incubation will save the animal. The antitoxin saves life under these conditions by neutralizing the toxin that has been irreversibly fixed by the motor neurons and the motor end-plates. The long-held belief that an impenetrable brain-blood barrier stands in the way of the uptake of blood-borne tetanus antitoxin is therefore just as untenable as is the belief that blood-borne tetanus toxin is likewise unable, by its diffusive power, to pass through this assumedly impenetrable barrier.

In order that no misconception may arise in connection with the demonstrable prophylactic action, in experimental animals, of timely injections of antitetanic serum in preventing the inevitable consequences of a lethal amount of the fixed toxin, we shall so far anticipate

the conclusions of our next paper, on the limits of this prophylactic action, as to make the following brief statement: After a single truly lethal dose of the toxin, or after many lethal doses have once been fixed (and this fixation, as has been shown, is always accomplished in a relatively few hours), the prophylactic action of the largest possible amounts of antitetanic serum that can be injected will not save the animal, if the injection of the serum is delayed until the first symptoms of a descending tetanus are clearly in evidence.

It must not, however, be thought that a conclusion of this kind, that is based upon animal experiments in which all of the several factors that determine death are completely under our control, is directly applicable to human tetanus. In dealing with a case of general tetanus in man, in which none of the factors are under control, and in which there is now no way of learning whether a full lethal dose of the toxin, or less, has already been fixed in the body, no other course is open to the physician than to inject antitetanic serum in accordance with present usage.

4. Further experiments on the partition of toxin between the blood and lymph show that within eight hours after the intravenous injection of many lethal doses, an equipartition, in the sense that the blood and the lymph of the thoracic duct contain the same amount of toxin per cubic centimeter, is established between the two fluids. "Peripheral" lymph contains, however, only one-half as much toxin per cubic centimeter as "visceral" lymph. The "wash-out" procedure, when carried out in the proper manner, effects the removal from both the blood vascular and the lymphatic system of all but a negligible amount of the non-fixed toxin. During the bleedings, as the toxin pressure in the blood vascular system is being steadily lowered, the lymph-borne toxin diffuses through the exceedingly thin walls of the lymphatic capillary net-works, traverses the intervening connective tissue structures and enters the capillaries of the blood vascular system, whence it is removed in the bleedings. We have referred to this peripheral transfer as the "reverse passage" of toxin. The amount of lymph-borne toxin recovered by the "wash-out" procedure amounted in one case (Experiment X-16) to 88 per cent of that calculated to have been present in the cardiovascular system at the time of the first bleeding. The source of this surplus is the closed lym-

phatic system. There is no evidence at hand to show that the thin fluid layers of the minute "tissue spaces" retain toxin in its free and assayable state, the form in which it circulates in the blood and lymph. It would appear, rather, that the toxin is fixed upon the organized boundaries of the so-called "tissue spaces."

5. The reverse passage of the toxin that occurs in the course of the "wash-out" or viviperfusion experiments gives us a method of approximately determining the lymph volume of the closed lymphatic system of a normal resting animal. When the calculation is made on the basis of the toxin content of the thoracic duct lymph, a minimum value for the lymph volume is obtained, and when the lower toxin content of the peripheral (cervical trunk) lymph is made the basis of the calculation, a maximum value is obtained. The true value lies between the two, being nearer the first than the second. Although we cannot, in the absence of studies on the toxin content of the mixed lymph, give the exact lymph volume of an anesthetized animal, our experimental data indicate that the lymph volume of such an animal is roughly equal to its blood volume. The substitution of diphtheria antitoxin for tetanus toxin in our "wash-out" experiments would probably lead to a more accurate method of determining the lymph volume of animals, both in the resting and the active condition, for very minute amounts of this antibody can be assayed by the sensitive intracutaneous test. We must, however, postpone further studies on the determination of lymph volume because we are now occupied with other studies on tetanus.

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